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The Journal of Laboratory and Clinical Medicine

VOL. XII ST. LOUIS, MO., AUGUST, 1927 NO. 11

CLINICAL AND EXPERIMENTAL

THE AMEBA COUNCILMANIA LAFLEURI, ITS APPEARANCE AND CLINICAL IMPORTANCE*

BY RAWSON J. PICKARD, M.D., SAN DIEGO, CALIFORNIA

IT IS difficult to distinguish the different species of amebae parasitic in man. They are small, they vary in number from day to day, protozoal infestation is often multiple, and degenerated forms are common. Diagnosis from the nuclear structure is complicated by the frequency of mitotic figures. The reaction to staining changes with the fecal reaction. Repeated examination of stained preparations is often necessary for diagnosis, and this means the study of the stained cysts, because of their more constant morphology and greater specific difference. The method of choice is the examination of both the fresh motile amebae and that of the motile and encysted forms stained in iron-hematoxylin; as between one of the two examinations there can be no question that an accurate diagnosis must be based on study of the stained cysts, and that laboratories not equipped to do this should not attempt the diagnosis of the fecal protozoa.

But the difficulties are not all self created by a desire for rapid diagnosis, nor due to lack of experience of the microscopist, nor to the difficulties of the technic of wet-fixed smears. There is the difficulty of attempting to follow the texts of the authorities, and this because the description of the two well-known amebae is confused by including the description of a third less known species, which latter, once recognized, permits definition in the description of all three and the ready separation of them from each other. Wenyon[1] says that an ameba 15 to 20 mu or larger, very active, containing red blood cells and an indistinct nucleus is certainly Entameba histolytica, and "the general rule holds for practical purposes that an ameba with included red cells is E. his-

*Received for publication, March 8, 1927.

tolytica." A slow-moving ameba with granular pseudopodia, food vacuoles containing yeasts and bacteria but never blood cells, with an eight-nucleate cyst, is E. coli.

What then of the nearly 8 per cent of patients having intestinal symptomatology (Kofoid's figures)[2] in whom there is present an ameba with, as constant characteristics, the size, great activity, clear pseudopodia, and occasionally ingested red cells of E. histolytica, and yet which, like E. coli has an eight-nucleate cyst and, in the motile amebae, visible nuclei and food vacuoles which contain yeasts and bacteria as well as erythrocytes? Do these make up the indeterminate infection noted by Wenyon (1 per cent to 17 per cent) in his stool surveys as "Entamoeba sp. (?)." Certainly this ameba is neither the E. histolytica (dysenteriae) nor E. coli as defined by Brumpt[3] or Dobell and O'Connor.[4]

Before Kofoid and Swezy[5] described Councilmania lafleuri this ameba was mistaken in the motile stage for E. histolytica and in the encysted for E. coli. Previous to the period of careful observation and interest in the intestinal protozoa which resulted from the wartime surveys showing the world wide frequency of these infections in all classes of the population and their causation of low-grade illnesses, only these two amebae were generally recognized in the feces. Cysts were seldom sought or recognized in the clinical laboratories and stained preparations rarely attempted. At present with attention centered on the fecal protozoa as one of the sources of the focal infections more exact work is requisite and is more often done.

Councilmania has been included with the other amebae, as atypical, figures of this ameba appearing in the texts under other names (e.g., Brumpt, p. 97, Fig. 32, F, H). For the clinician Councilmania lafleuri is a new distinct species, as well defined as either of the others, once Councilmania is separated from them. Studying the abnormal shapes of the cysts, finding many of them to all appearance budding in the stools, staining the nuclei and finding them to be of a different type from those of the other amebae, one might concede its status as a new genus, as stated by Kofoid, as readily as one concedes a genus for Endolimax nana; the least one could do would be to classify it as a new species of the Entamebae, differing from the other two as much as they differ from each other. One cannot agree with the texts that Councilmania is a "synonym of E. coli." To this confusion of description in which to the morphologic forms of the two Entamebae there are added as exceptional, the forms that are constant in the third species (Councilmania), which once separated furnish three clear pictures, some texts add an additional obscurity by purposely passing over details in a work where attention to detail is everything. What criticism is there for the remark that attention to details of nuclear structure "may assist" in a diagnosis? If Kofoid is in error in insisting on details that it is impossible for the less expert clinician to demonstrate, his is at least the error of setting a high standard.

Councilmania lafleuri was described by Kofoid and Swezy in 1921 and the authenticity of the species, as evidenced by the constancy of the group of characteristics distinguishing it, should be apparent to any microscopist. Yet this ameba is not yet generally recognized. Wenyon heads his notes on it "an

aberrant form of E. coli,'' and thinks it would be quite impossible in ordinary work to distinguish it, either vegetative or encysted, from E. coli. There is only one reply, and that is that ordinary work is not at all acceptable for stool examinations in this country today; it will not separate the small races of E. histolytica from E. nana, for instance. At the same time it is true that Councilmania much resembles the two Entamebae, nor do they so greatly differ from each other that one can determine the species of each individual seen on a slide. The chief difference from E. coli in the motile amebae is the active movement, clear pseudopodia, and the occasional ingestion of blood cells, from E. histolytica the large vacuoles containing yeasts as well as blood, and the visible nucleus. The cysts differ from those of E. coli in the larger karyosome consisting of several granules, and the numerous irregularly shaped cysts, ellipsoidal rather than spherical, and the prebudding and budding forms, a character not found in other cysts. Small free amebae are not uncommonly found near the cysts of Councilmania, and Kofoid has observed the buds emerge on the fresh slide,[2] as well as seen them in feces fixed in paraffin where the question of pressure and rupture was absent. Except for an observation of Wenyon who saw protrusions from cysts of ''E. histolytica (?)'' ''possibly representing the escape of small amebae from the cyst,'' no one has seen the budding of other entamebae in the intestine, although they must bud in a new host, but under conditions as yet not reproduced. The cysts of irregular form, and in which budding was seen, were so carefully described by Mathis and Mercier[13] that we can recognize them as Councilmania, although they wrongly were considered to be schizogonic forms of E. coli, an interpretation due to the fixation in sublimate, which does not reveal the cyst wall in Councilmania except in those cysts, evidently destined for another host, in which the wall is thick and no tendency to bud is seen. Fixed in Bouin's solution following Langeron's technic the wall is always visible due to the retraction of the cytoplasm. It is easy to convince one's self as to the meaning of the budding cysts by search over a stained slide. Not being a protozoologist I cannot have an opinion on Wenyon's hint that Councilmania does not present sufficient difference for it to be placed in a new genus. True, the cysts of E. histolytica and E. coli never bud within the bowel, but these cysts must bud sometime; perhaps the site and time of gemmation have had too great importance given to them on account of their absolute diagnostic value. There can be no doubt as to the specific existence of this ameba. Hall and Reed[6] for instance had no difficulty in separating the ''cases in which E. coli of the Councilmania type was harbored'' in their study of the pathogenicity of these two amebae.

We must not bind ourselves to facts so readily observed because of the delayed acceptance of this ameba by the writers of the textbooks, for the facts resolve a not infrequent perplexity into clarity, once they are aligned as seen and not forced into the two old cadres.

We infer from the presence in monkeys of species with identical appearance that E. histolytica and E. coli have been long present in man and the primates. Councilmania, if the presence of species of the genus is a proof, may have come to man from the rodents, as Kessel[7] suggests, or gone to them from man whom they have long molested. One might wonder whether this ameba,

hard to distinguish in the active state from the ameba of dysentery, and in the cyst from E. coli, might not be a more primitive species linking the two, showing the history of E. histolytica evolving into a tissue parasite and the development of the colon ameba as a lumen parasite; whether clear ectoplasm and active pseudopodia are structural modifications of an ameba that feeds on the tissues of its host, perhaps forming a capsule that protects the endoplasm and nucleus from the destructive effects of the histolytic enzyme.

Kofoid found Councilmania as an infection in 7.8 per cent of 4763 persons referred to him for examination, most of them patients under a physician's care; there were 3.8 per cent more with E. coli, a total incidence of 11.6 per cent. The patients referred to my laboratory for fecal examination have nearly all been sent on account of symptoms of chronic amebiasis. Chronic infection with intestinal protozoa would more accurately describe their semeiology. Among the last 43 patients there were 21 with negative feces, with a total of 143 stools examined, and in the 22 patients who had intestinal protozoa there were 30 infections; 10 with Chilomastix, 3 with Trichomonas, 3 Giardia, 1 each of E. coli, Dientameba fragilis, and a small undiagnosed ameba, probably Endolimax nana, 8 with E. histolytica of whom 3 had dysentery. Of these two had never lived where dysentery is endemic. One was an eight-year old native of San Diego infected with the small race (stained: cysts 4 mu to 5 x 6 mu, vegetative 8 mu in diameter), the other, aged 45, had lived in the northern midwest and in California.

There were three infections with ameba of the species described by Kofoid as Councilmania lafleuri. Slides from each were sent to Dr. Kofoid who confirmed the diagnosis.

CASE 1.—Mrs. P., about fifty years of age, had lived in Colorado before coming to California four years ago, partly for her health, her complaint being weakness, "spells of faintness," and constipation. The last had become obstinate two years previously when an operation was advised at which adhesions were found obstructing the ileum. After a few months the fainting spells recurred with loss of strength and constipation. At this time the fex was examined, numerous Councilmania were found, both motile amebae containing blood cells, cysts and budding cysts, along with dientameba and another (?) small ameba. No other cysts were found. Treatment with stovarsol (not well borne), alternating with emetine, cleared the protozoa so none could be found in a series of stools four months later. There has been practically no clinical change, but the time elapsed is too short to judge.

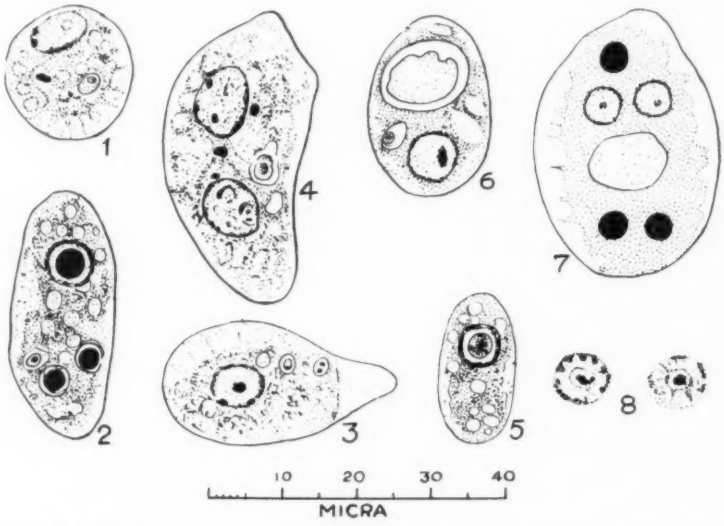
CASE 2.—Mrs. B., in her forties, had always lived in the northern midwest and California. For four years she suffered from a fatiguability which kept her in bed much of the day, with pains in the sacrolumbar region, which were diagnosed "toxic" after many thorough examinations. There were found in her stool Chilomastix, a few small amebae not positively identified (E. nana?), and numerous Councilmania, motile, encysted and budding. Six weeks after her last treatment with stovarsol in a series of ten stools in nine days, several following salts, it was impossible to find any protozoan. Her condition was definitely improved, and now three months later, her stools are still negative. She feels well and considers herself completely cured.

CASE C.—Miss S., aged forty years, had a secondary anemia (70% Newcomer) that persistently recurred after treatment. In the search for a cause, after several years, the stool was finally examined with the finding of Trichomonas, Chilomastix, a few small (9.5 mu) E. histolytica but no cysts, and numerous Councilmania lafleuri. The Councilmania had a nucleus that was markedly visible, as noted below. There were numerous cysts, budding

cysts, a few small escaping amebae 4 to 5 mu in diameter. A month after treatment which had been followed by great improvement, she noticed beginning loss of strength and re-examination showed numerous Councilmania, alone. With return to stovarsol treatment she is again clinically well and active in her profession.

CHARACTERISTICS

Fresh.—Large numbers of actively motile amebae were present varying in size from 12 x 15 mu to 55 x 20, average about 20 x 30, with perfectly clear hyaline ectoplasm from which the pseudopodia were formed. In two cases the endoplasm was vacuolated, somewhat granular, greyish, but less dense than E. histolytica, as the nuclei were visible. In Case 3 the endoplasm was but slightly denser than the ectoplasm, and in the fresh feces the nucleus was conspicuous among the food vacuoles as a circle (optical section) of bright, refractile granules. After the first course of treatment in this case the body of



Figs. 1 to 6.—Councilmania lafleuri, vegetative. Fig. 4 has two nuclei, Fig. 2 contains two red blood cells.

Fig. 7.—Binucleate E. histolytica containing three blood cells, to show difference of ectoplasm in stained specimen.

Fig. 8.—Nuclei of C. lafleuri, vegetative.

the ameba became still clearer and less dense so that the ameba was hard to distinguish under the lower magnifications and except with dim light. Food vacuoles were conspicuous in all cases, containing yeasts, bacteria, and in 15 to 30 per cent blood cells. Motion was active, the pseudopodia being suddenly formed like those of the dysenteric ameba. The nucleus changed shape during motion. The cysts, 15 to 20 mu in diameter, were thick walled. The eight nuclei were readily seen in iodine-eosin stain. Their shape was irregular. Budding and pyriform cysts were as common as spheroids.

Stained.—Motile forms. In preparations wet fixed in either hot or cold alcoholic Bouin's solution, and stained in iron hematoxylin, the vegetative amebae were usually caught in movement and fixed as round ended cylinders, or with one end stretched out in a long neck. The reaction to stain varied on

the same slide. The endoplasm of some amebae took so deep a stain that the nucleus was obscured and with the clearer vacuoles the ameba looked like a purplish sponge. The ectoplasm did not often show the distinct demarcation one might expect from the appearance in life; when stained it appears not like the sharp bordered fine foam-like ectoplasm of E. histolytica, but as irregular lighter vesicular areas on the sides or ends of the ameba, pointed processes of the endoplasm flowing through out to the wall or toward it (Figs. 1 to 6). When present in the ameba of dysentery they terminate more sharply (Fig. 7). In about 25 per cent when stained the ectoplasm was still distinct (Figs. 1, 3). Blood cells (Fig. 2) were present in about one-third (of 96 amebae), yeasts, bacteria and larger unrecognizable fragments (Fig. 6) were in the vacuoles. The stained nuclei varied greatly in the amount of chromatin on the nuclear membrane. In about half (motile) the karyosome was large, over 1 mu. A clear area, the halo, was exceptional about the karyosome. The linin threads were frequently radial when the karyosome was small. (Fig. 8, isolated nuclei).

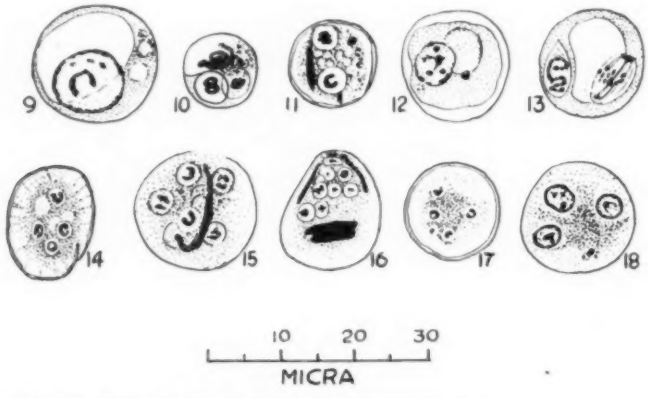
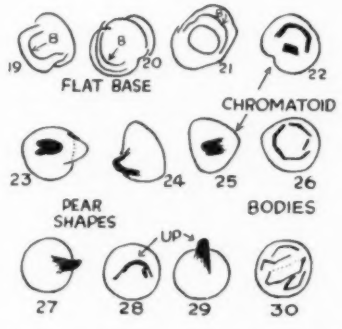


Fig. 9.—Mononucleate cyst, C. lafleuri, large glycogen vacuole.
Fig. 10.—Binucleate cyst, glycogen vacuole.
Fig. 11.—With chromatoids.
Figs. 12 and 13.—Nuclei in mitosis.
Figs. 14 to 17.—Eight-nucleate cysts. Figs. 15 and 16 contain chromatoids.
Fig. 18.—Four-nucleate cyst in mitosis.

Stained Cysts.—The cysts stained irregularly, frequently taking a deep "chromatoidal" stain, like many of the active amebae. These when de-stained lost the stain from the nucleus. Cysts averaged about 17 mu in diameter. Some were pyriform (Figs. 23, 24, 27, 28, 29), many cysts had irregular folds (alcoholic Bouin fixation) and rested on the slide upon a flatter area, as seen by focussing (Figs. 19, 20, 21), or with a neck or bud pointing up to one side (Figs. 23, 24, 28, 29) forming a figure not readily drawn as seen, but obviously not the product of "pressure of the cover glass," "rupture," etc. I found a few cysts that might have been "ruptured." I so thought because the herniated cytoplasm (Fig. 32) contained neither chromatoids nor a nucleus, and took a deep counterstain of eosin, which was not true of the gemmae. Whatever the cause of these ruptures, and there is no evidence that hot fixation ruptures, they took place through a point in the cyst wall that it was not unreasonable to suppose was prepared to give exit to a bud. I found nothing

that could be considered the crushing of a cyst by the cover glass, an experiment I have not succeeded in performing. Often buds were found in the thick areas where focussing showed the cover well above the cyst, resting on debris. Buds were often found on the upper side of a cyst (Figs. 31, 35, 36), and the pear-shaped cysts usually were fixed on the slide with the neck up, a position naturally taken by an irregular body with a large base, and impossible under pressure. The gemmation cannot be an artefact.

Cysts fixed in hot Schaudinn's solution are more regularly spheroidal or ellipsoidal, are farther shrunken from the fecal debris, and present a more evenly distributed cytoplasm (Figs. 15, 17), than those fixed in alcoholic Bouin's solution, either hot or cold. In this fixative the cyst wall leaves a narrower clear area between it and the bacteria round about (Fig. 42, photograph) than with Schaudinn's. With Bouin's often a denser cytoplasmic mass, with the nuclei, is drawn away from the wall, leaving a space perhaps 2.5 mu wide crossed by fine protoplasmic threads, giving the area the light, vacuolated appearance of the ectoplasm in the motile Councilmania (Figs. 14, 31, 33, 34, 35, 37, 42). But this method of fixation brings out another interesting fixture.



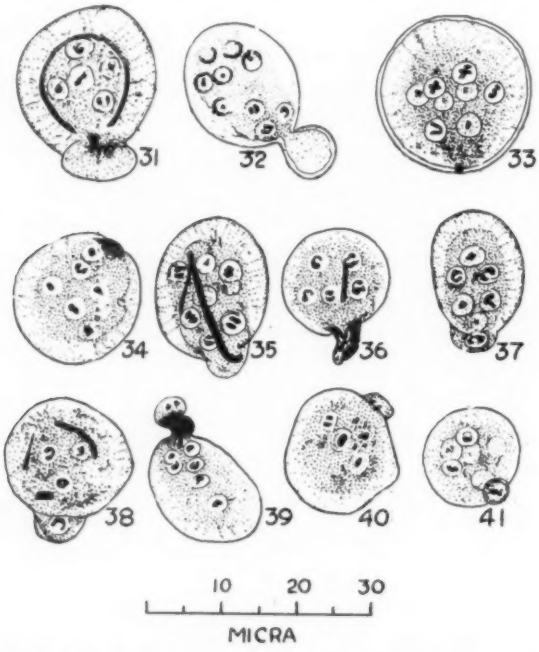
Figs. 19 to 30.—(Not drawn to scale.) Irregular contours of cysts at different levels of focussing are shown in Figs. 19 to 21. Figs. 22 to 30 show chromatoids of different shapes. Figs. 23, 24, and 28 show pear-shaped cysts, in Figs. 24 and 28 with chromatoids in neck of pear. Figs. 27 and 29 have pointed chromatoidal extrusions.

The nuclei and surrounding denser cytoplasm are not always suspended in the center of the cyst. The mass attaches to a point on the cyst wall, where may be seen an irregularity, either a dark line through the wall, a "pore" (Fig. 33), a depressed area (Fig. 34), apparently a break or thinning of the wall, a bulging of the cyst (Fig. 16), or the formation of a pyriform neck, or actual gemmation (Figs. 31, 35 to 41). The tendency to bud is thus seen in cysts not yet budding, and the attachment of the cytoplasm to a part of the wall often morphologically marked shows the frequency and the natural occurrence of gemmation in this species of ameba.

I found budding present in 20 to 40 per cent of the cysts, in about 3 per cent there was a small ameba with one nucleus separate beside the mother cyst, with 7 or 6 nuclei remaining (Figs. 41, and 42 photograph). Budding cysts often show a massing of nuclei near the bud, chromatoid material was often present in the extrusion ahead of the nuclei (Figs. 16, 24, 27, 28, 29, 31, 34, 35, 36). Sometimes the budding process is intensely stained, the border fading toward the cytoplasm (Figs. 29, 39). The chromatoid matter may be in sharp

fragments like crystals, or in threads (Fig. 30). In a few cysts I have found the chromatoidal staining ridge as described by Kofoid. In such a cyst if there was a bud it was situated on the ridge, which lies on the denser cytoplasm within the cyst wall (Figs. 15, 16, 31).

The frequency of chromatoid bodies or chromatoidal staining cytoplasm in the buds in the eight-nucleate cysts, and the comparative infrequency of chromatoidals in cysts in which there was an extrusion of cytoplasm containing a nucleus, a new forming ameba, raised a question as to the purpose of the extrusion of the chromatoidal material and the sequence of these events. It might be that the material staining like that of the chromatin of the nuclei is condensed from the cytoplasm previous to and as a part of the process of gemmation, and that the cyst which is about to break up into small amebae first con-



Figs. 31 to 40.—Eight-nucleate, except Fig. 33. (All nuclei not in focus.) Fig. 31, bud with chromatoidal matter, chromatoidal ridge encircling cytoplasm. Fig. 32, bud without nucleus, cyst with nine degenerate nuclei. Fig. 33, cyst with "pore." Fig. 36, chromatoid extrusion in bud. Fig. 39, same with nucleus in addition.

Fig. 41.—Seven-nucleate. Small free ameba beside cyst.

centrates and extrudes the chromatoid material. Perhaps this is the nuclear food supply. It is then followed by the nuclei which must leave the cyst to survive, breaking through the cyst wall in gemmation, each surrounded by a small mass of the "hungry" cytoplasm, and forming small active amebae. The formation of the chromatoidal bodies appears thus to be a stimulation to gemmation.

The nuclei in the ripe cysts, about 2.5 mu in diameter, number eight, and have seldom any chromatin on the membrane. The nuclei appear as lighter areas in the cytoplasm, sharply defined, with the karyosome as a group of chromatin granules in a mass, circle, V-shaped, or irregular arrangement.

Mononucleate cysts (2 per cent, Fig. 9), and binucleate cysts (5 per cent, Figs. 10 to 13) usually have a large glycogen vacuole, resembling those of E. coli. Wenyon suggests that the cysts with glycogen are terminal, never forming 8 nuclei; a dividing nucleus like that in Fig. 13 would seem to be growing. Fig. 18 is a four nucleate cyst (3 shown) in mitosis. About 2 per cent had 4 nuclei. Those with 5, 6, 7, have budded. The cyst shown in Fig. 32 had nine.

Further details can be found in the papers by Kofoid and his associates, and by following their technic, pictures similar to their figures can be seen.[8] I have given above such details as seemed different with my preparations. I feel that the technic of wet fixation in alcoholic Bouin's solution is much simpler than Schaudinn's and gives as good detail, although different in some particulars. This method appears more practical for the average laboratory where economy of time and space are essential, and where stool work is only one of a large number of technics to be followed each day. With this method it is possible, in the press of work to leave a process temporarily incompleted. Theoretically, as Langeron has pointed out, the alcoholic picroformol with acetic acid is an ideal fixation.[10]

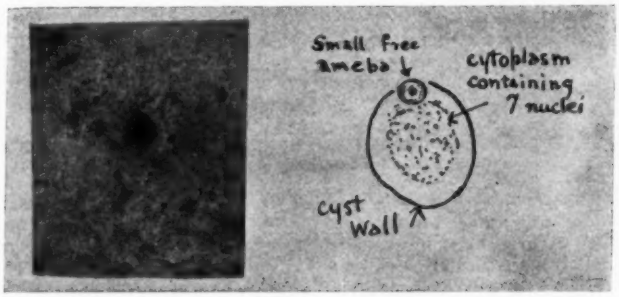


Fig. 42.—Photograph of seven-nucleate cyst with small ameba free beside it, sketch to demonstrate.

All figures except Fig. 15 are from preparations wet-fixed in alcoholic Bouin's solution, and stained iron hematoxylin.

I fix slides in alcoholic Bouin's solution, cold, for an hour, or hot, at about 60° C., for five minutes, then follow with 95 per cent alcohol for hardening, following the technic for iron-hematoxylin given by Langeron in his *Précis de microscopie*. For cold fixation the flat glass "Laveran" boxes are best. For the rest of the procedure Coplin or Borrel jars will serve. Half as large a set-up is needed for Bouin fixation. One may clear from the 95 per cent alcohol to balsam with creosote where absolute alcohol is difficult to obtain. I seldom get a good stain when in haste.

The ameba Councilmania seems to be pathogenic in the manner that the small races of E. histolytica are pathogenic, causing low-grade neurotoxic disorders and constipation. The cases reported by Kofoid and Swezy were under the care of physicians for symptoms referable to the intestinal tract, "bowel conscious" as Boyers puts it,[11] or were subnormal physically; the three reported here were all invalids. Hall and Reed report 11 cases with E. coli, and 16 with Councilmania, with "neurasthenia," epigastric discomfort, flatulence, constipation, neuralgic pains. On treatment and eradication of the amebic infection

TABLE I

TABLE OF DIFFERENTIAL CHARACTERISTICS*

INDIVIDUALS	C. LAFLEURI NUMEROUS	E. HISTOLYTICA NUMEROUS	E. COLI FEW
MOTILE AMEBAE, UNSTAINED			
Occurrence in "bowel conscious" patients	About 10 per cent	About 10 per cent	About 10 per cent
Size	(10x15) 25x35 mu (35x65)	(5) 20-30 mu	(15) 20-30 mu
Appearance	Greyish, nucleus visible	Bluish, refractile, n. invisible	Greyish, n. visible
Ectoplasm	Distinct, glassy	Distinct, glassy	No demarcation
Pseudopodia	Clear, broad, single	Clear, several	Granular
Endoplasm	Vacuolated	Granular, uniform	Vacuolated
Food	Bacteria, yeasts, blood cells	Blood cells, feeds by absorption†	Bacteria, yeasts, never blood
Motility	Very active	Very active (small races sluggish)	Sluggish
MOTILE AMEBAE, STAINED			
Ectoplasm	Distinct, or retuculated, with indeterminate border	Clear, distinct	No demarcation
Nuclear membrane	Thick, chromatin granules fine or coarse	Thin, chromatin granules fine	Thick, chromatin in coarse granules
Linin network	Often radial	Radial threads	Irregular, may hold chromatin
Karyosome	Over 1 mu massed, no halo	0.5 mu, rare 1. Small central granule, with clear halo	1 mu. Single granule often eccentric
CYSTS STAINED			
Size	16-20 mu	(6) 7-12 (20)	(10) 15-20 (30)
Wall	Thick, cyst hard to stain, 1 mu	Thin, ½ mu	Thick
Shape	Irregular, spheroidal, pyriform, depressions	Spheroidal	Spheroidal
Nuclei	1-2 per cent, large glycogen	1-30-45 per cent	1- rare
	2-5 per cent large (vacuole)	2-13-30 per cent	2- rare
	4-2 per cent	4-25-55 per cent	4- rare
	8 common		8- common
	16 rare		16- rare
Peripheral chromatin	None, or thin	Thin, even	Large beads, plaques
Karyosome	Massed or dispersed granules	Single central bead	Single eccentric bead
Chromatoid material	Blunt, sharp pointed or threads, or diffuse edge. Contributes to buds. About 30 per cent	Large blunt masses, 50 per cent	Sharp V 10 per cent splinterlike
Glycogen	Large in precystic, 1, 2 n. vacuole	Diffuse, not abundant	Largest vacuole in binucleate cysts
Budding	10-20 per cent, small amebae, 3 per cent	Unknown	Unknown
Fresh, unstained	Nuclei visible in iodine only	Nuclei visible in iodine only	Nuclei faintly visible

*Compiled from Brumpt, Dobell and O'Connor, Kofoid, and Wenyon.
†The small races of E. histolytica often contain bacteria.

two-thirds were definitely improved, 10 per cent greatly improved. The infections are usually of years' duration, and form, as Bowers insists, but a part of many infectious and degenerative disorders. Improvement is all that can be expected, and a year is not too long to wait for it. Like chronic amebiasis

with E. histolytica the infection causes symptoms in midlife and beyond. If there is a well-defined effect, like the anemia in Case 3, a result can be expected in a short time. If Councilmania, like E. histolytica, infects the gall bladder, as seems likely,[12] it may penetrate to tissues still farther from the intestine.

Kessel thinks the two species of ameba in rats and mice classified by him in the same genus and probably not pathogenic, but in his experiments he found evidence of immunity, which certainly does not exist against a wholly innocuous parasite. Then, it is difficult to recognize a low-grade illness in a rodent.

In the volume of literature about the parasitic amebae of man a few attempts have been made to show that E. coli is sometimes other than a harmless feeder on the bacteria and yeasts in the intestinal lumen. There have been occasional reports that E. coli ingested red blood cells, and seemed to have assumed an actively pathogenic rôle, but they were scattered observations, and of course lacked the proofs that are demanded properly of those who wish to show the pathogenicity of a bacterial species. There was, however, sufficient to arouse suspicion either that E. coli could under certain conditions become a tissue feeder, or that there might be a pathogenic species confused with it.

If Councilmania lafleuri, Kofoid and Swezy, is not an acceptable species, then Entameba coli must be accepted as becoming pathogenic at times, undergoing at the same time structural changes that are pathognomonic of the change, and permanent. If the goddess of science has a form of worship it is the religion of doubt. It is therefore to be expected that a new species, like all new findings, must wait for recognition. But if a species is a group of similar organisms readily distinguished from other groups, Councilmania lafleuri is a valid species.

REFERENCES

[1]Wenyon: Protozoology, Wood and Co., New York.

[2]Kofoid, Swezy, and Kessel: On the Distinctions Between E. Coli and Councilmania Lafleuri, U. Calif. Pub. Zool., 1924 xxvi, No. 3, 21-39.

[3]Brumpt: Parasitologie (new edition to appear May, 1927), Masson, Paris.

[4]Dobell and O'Connor: The Intestinal Protozoa of Man, Wood and Co.

[5]Kofoid and Swezy: Councilmania Lafleuri, a Parasitic Ameba of the Human Intestine, U. Calif. Pub. Zool., June 23, 1921, xx, No. 7.

[6]Hall and Reed: Entamoeba Coli, a Clinical Study, Jour. Am. Med. Assn., June 20, 1925, lxxxiv, 1914-15.

[7]Kessel: Amebae of Culture Rats and Mice, U. Calif. Pub. Zool., Feb. 20, 1924, xx, 489-544.

[8]Kofoid, Swezy, and Kessel: On the Genus Councilmania, U. Calif. Pub. Zool., Oct. 31, 1923, xx, 431-445.

[10]Langeron: Precis de Miscroscopie, (Theory and Best Practical Methods) Masson, Paris. Stool technic only in "Coprologie miscroscopique," Langeron and Rondeau du Noyer, Paper, Masson.

[12]Kofoid: The Occurrence of Cysts of Councilmania in the Duodenal Drainage, Proc. Soc. Exper. Biol. and Med., 1926, xxiii, 299-301.

[11]Boyers, Kofoid, and Swezy: Chronic Human Amebiasis, Jour. Am. Med. Assn., Nov. 7, 1925, lxxxv, 1441-1447.

[13]Mathis and Mercier: La schizogonie chez les entamibes intestinales, Bul. Soc. Path. Exotique, 1927, x, 311.

TRYPTOPHANE REACTIONS IN THE SPINAL FLUID*

By Burnham Sarle Walker, Ph.D., and Francis Harper Sleeper, M.D.

IN AN attempt to apply the Liebermann-Burchard test for cholesterol directly to the spinal fluid, Boltz[1] observed a color reaction in the fluids of pareties and of some cases of other forms of neurosyphilis. No explanation for this color development was at hand, but on account of its almost uniform incidence with fluids of pareties and tabetics, and its equally uniform absence from the fluids of mental patients with other than a syphilitic condition, it was offered tentatively to the profession as a test of apparent diagnostic value in these neurosyphilitic cases.

The technic of the test as published is as follows: to 1 c.c. of the fluid to be tested is added 0.3 c.c. of acetic anhydride, drop by drop, with shaking. To this mixture is added in a similar manner, 0.8 c.c. of concentrated sulphuric acid. After five minutes of standing, the tube is examined against a white background, and the appearance of a blue or lilac color is considered as a positive reaction.

As a result of the failure to obtain cholesterol reactions with other standard tests, Boltz concluded that the chromogen in this test was not cholesterol, but was some other substance which appeared, or increased in amount, in neurosyphilitic conditions.

Grossman[2] made a clinical study of the test, showing that it coincided with the Wassermann and other tests used in the diagnosis of general paralysis. He also made the interesting observation that a solution of egg white in water would give a "positive" reaction. In spite of this, however, he interpreted the reaction in terms of minute quantities of cholesterol.

Harris[3] also studied the test clinically. Out of 92 cases of general paralysis whose fluids he examined by this method, 89 presented a positive reaction. Two out of five cases of other forms of neurosyphilis were positive. Of other types of mental disease 83 cases were investigated, and only one of these showed a positive response. Harris points out that the agreement here is nearly as good as that obtained with the Wassermann. In regard to the explanation of the reaction, he also inclines to the belief that cholesterol is the substance involved.

A hint as to the nature of the reaction is given us by the work of Aiello[4] and of Brugi,[5] published in Italy. They investigated the spinal fluid with the purpose of detecting and estimating the quantity of tyrosine and of tryptophane present. For tyrosine they used the familiar Millon reaction, and found that the amount of tyrosine found paralleled the amount of protein. Their method of estimating tryptophane was based upon a modification of Voisinet's method proposed by Fürth,[6] and both Aiello and Brugi seem

*Contribution from the Evans Memorial and Worcester State Hospital.
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to feel that by using this method they are measuring free tryptophane, and not the combined tryptophane in the protein molecule. The experiments are not reported in detail and it is difficult to evaluate their exact import. At any rate, the Fürth-Voisinet reaction for tryptophane was negative in almost all fluids except those of tubercular meningitis.

The observation of Grossman[2] that the Boltz reaction was positive with egg-white solution led the present writers to consider that the Boltz reaction might also be an index of excess protein in the spinal fluid. With this end in view, the egg-white experiment was repeated using different strengths of commercial egg albumen, standardized by means of nitrogen determinations by the Kjeldahl method. It was found that egg albumen solutions containing 30 mg. or more protein per 100 c.c. gave a positive reaction, increasing in intensity with increasing amounts of protein. A solution containing 15 mg. protein per 100 c.c. gave no test whatever with the Boltz reagents, whereas solutions intermediate to these values gave a slight color, which was difficult to apprehend with certainty.

From these considerations, it becomes apparent that we are dealing in the Boltz reaction with a test for protein or for some constituent of protein. Reference to the work of Hopkins and Cole[7] gives us very definite information in this respect. Aldehydes are present as impurities in all ordinary samples of glacial acetic acid and of acetic anhydride. In the presence of concentrated sulphuric acid the aldehydes react with the tryptophane groups of any protein which contains such groups, producing the characteristic lilac or blue color (the Adamkiewicz reaction). Hopkins and Cole found that one of the aldehydes present in glacial acetic acid or acetic anhydride is glyoxylic acid, and that solutions of this acid or its salts would give the characteristic reaction.

Hence, to verify our conclusion that the Boltz reaction is a test for protein, we substituted for the acetic anhydride of the original Boltz procedure, a solution of the magnesium salt of glyoxylic acid, prepared according to the method of Benedict[8] and obtained positive results with paretic fluids and with egg albumen solutions.

Since the intensity of the color developed in the reaction was found to be proportional roughly to the amount of protein present, it was possible to prepare standard solutions, the color of which could be used as a basis for classifying the reactions obtained from spinal fluids. Thus, the color obtained with a solution of egg albumen containing 30 mg. per hundred c.c. (standardized by Kjehldahl determination of total nitrogen) was designated as +. A solution twice this strength gave a somewhat stronger color, called ++, and a solution containing 120 mg. protein per 100 c.c. was taken as the +++ standard. Since the majority of the fluids studied did not reach this strength, and none surpassed it, it was found unnecessary to continue the standards beyond this level.

To check the results of the Boltz test against the actual amount of protein in the fluids under investigation, the protein in these fluids was determined by a modification of the Folin micro-Kjehldahl method, suggested by the work of Ling.[9] One c.c. of the fluid was measured into a 50

TABLE I

MACRO-KJEHLDAHL MG. N PER 100 C.C.	PROTEIN PRECIPITATION AND DIGESTION	DIRECT DIGESTION
10.4	10.1	10.6
30.2		30.2
20.3		21.4
5.2	5.3	

c.c. pyrex centrifuge tube, and about 7 c.c. of water added. To the diluted spinal fluid was then slowly added 1 c.c. of the sodium tungstate solution and 1 c.c. of the 2/3 normal sulphuric acid, these solutions being the same as are used for the precipitation of proteins in the Folin-Wu system of blood analysis. The tubes were then allowed to stand, after mixing, for an hour or more, and then centrifuged for five minutes at a moderate speed. The supernatant liquid was poured off the precipitated protein, and the tubes

TABLE II*

PATIENT		PROTEIN	NPN	BOLTZ	WASS.	GOLD CURVE	DIAGNOSIS
1		167	14.8	+++	pos.	5555543000	General paralysis
2		120	13.2	+++	pos.		" "
3		77	19.7	+++	pos.	5444330000	" "
4		61	12.8	++	pos.	1122332100	G. P. (malarial treatment)
5		60	12.2	+	pos.		General paralysis
6		59	25.1	++	pos.	5543000000	" "
7		55	22.8	++	pos.	5555543321	G. P. (cardiorenal complications)
8		47	21.1	+	neg.	0012210000	Involutional melancholia
9		45	17.2	++	neg.	3422100000	Cerebrospinal syphilis
10		44	20.3	+	neg.	0000110000	Not diagnosed
11		42	9.5	+	neg.		Dementia precox
12		42	13.7	+	neg.	1110000000	Manic depressive, manic phase
13		41	11.9	±	neg.		Senile psychosis
14		38	14.8	+	pos.	5543200000	G. P. (malarial treatment)
15	(Dec. 8)	38		+	neg.	0000000000	Manic depressive,
15A	(Dec. 1)	37		+	neg.	0033320000	manic type
16		37	13.4	+	neg.	1122000000	Undiagnosed psychosis
17		36	13.5	+		0012000000	Unclassified psychosis
18		35		+	neg.	0000000000	Dementia precox
19		33	8.8	±	neg.	0000000000	" "
20		31	11.9	+	neg.	1221000000	D. P. catatonic
21		29	14.0	+	neg.	1112000000	D. P. paranoid type
22		28	14.8	±	neg.	0001100000	Not diagnosed
23		27		+	neg.	0000000000	Senile psychosis, paranoid type
24		27	12.9	±	neg.	0011000000	Not diagnosed
25		26	20.3	±	neg.		Epilepsy, syphilis
26		24	21.5	±	neg.		Psychosis; alcoholism ch. deterioration
27		20	20.9	+	neg.	0001110000	Not diagnosed
28		19	13.2	+	neg.	0011000000	Not diagnosed

*The figures for protein and for nonprotein nitrogen are in mg. per 100 c.c. spinal fluid.

inverted over a filter paper until dry. The precipitate formed was then digested in situ with 1 c.c. of Folin's undiluted digestion mixture, exactly as in the determination of the nonprotein nitrogen of the blood except for the stronger acid. After digestion the contents of the tubes were washed into 100 c.c. volumetric flasks and Nesslerized with 30 c.c. Nessler's solution, the excess being necessary to neutralize the highly acid digestion mixture. The standards for color comparison were 0.05 mg. and 0.10 mg. nitrogen in the

form of ammonium sulphate. One c.c. of the digestion mixture was added to each flask containing standard solution, and 30 c.c. Nessler's used as in the unknowns.

Simultaneously with the above determination of the protein nitrogen a micro-Kjeldahl was run on a 1 c.c. sample of the spinal fluid to determine the total nitrogen. By difference of these values it was then possible to calculate the nonprotein nitrogen.

The accuracy of the above method of determination of the protein nitrogen was checked by determinations of the protein nitrogen in egg albumen solutions which had been standardized by macro-Kjeldahl nitrogen determinations.

Table II gives the results of our determinations on spinal fluids, arranged in order of decreasing amounts of protein found.

From inspection of Table II it becomes evident that there is a definite correlation between the amount of protein present and the intensity of the Boltz reaction. Thus the mean of the protein values of the three +++ cases is 121 mg. per 100 c.c., which corresponds exactly with the egg albumen standard of 120 mg. per 100 c.c. The mean of the four ++ cases is 55 mg. per 100 c.c., again agreeing closely with the standard of 60 mg. Similarly, the mean of the 16 cases reacting + is 36 mg. per 100 c.c., with 30 mg. as the standard.

An exact agreement is hardly to be expected, since egg albumen does not exactly agree with serum albumen or serum globulin in tryptophane content, and the protein of the spinal fluid has been known for some time to be a variable mixture of albumen and globulin. Fürth[6] gives figures showing that the tryptophane contents of these substances are not widely divergent, however.

Egg albumen contains	2.6 per cent tryptophane
Horse serum albumin	1.2-1.5 per cent
Horse serum globulin	4.1-4.7 per cent

Thus it happens quite fortuitously that egg albumen, occupying a median position in regard to tryptophane content between albumin and globulin, makes an ideal standard for the rough determination of mixtures of the two.

The doubtful cases (those indicated by the sign ±) fall into two categories: some are weak reactions, where the color was so faint as to be difficult of apprehension, others are reactions where some interfering substance in the fluid caused the development of a brown color which masked the lilac tint of the reaction. One possible interfering substance would be sugar in excess; it was found that the addition of a few drops of 1 per cent glucose solution to the standard egg albumen solution before performing the test would cause such a brown coloration to appear upon the addition of sulphuric acid.

In no case did we obtain a frankly negative reaction. There was always some change in the color of the fluid upon the addition of the reagents, and in most cases this could be seen as a pink or lilac tint. The only cases where this was not observed were those in which the development of the brown color

took place. This is compatible with the knowledge which we have as to the presence of protein in the spinal fluid, some protein being always present, which gives the faint reaction. In general paralysis and in other conditions where the protein content of the spinal fluid is increased we find a marked increase in the color development with the Boltz reagents.

From these considerations we must conclude that the Boltz reaction, used in conjunction with standards made up from egg albumen solutions of known protein content, is of value in roughly estimating the amount of protein in the spinal fluid, and that it cannot be considered as being strictly specific for general paralysis or even for neurosyphilis, inasmuch as extreme increases in spinal fluid protein occur in other conditions, e.g., typhus fever, and certain cases of disseminated sclerosis, to choose from widely separated fields.

SUMMARY

1. The Boltz reaction has been shown to be dependent upon the tryptophane present in the protein molecule.

2. It has been demonstrated as varying in intensity with the amount of protein present in spinal fluids and in egg albumen solutions of known protein content.

3. This relationship has been found not to be strictly quantitative, as a result of varying tryptophane content and of the presence of interfering substances in varying quantity.

4. It is suggested that the Boltz reaction may be of considerable value in estimating increases in the protein content of spinal fluid, although it cannot be considered as specific for any single clinical entity.

REFERENCES

[1]Boltz: Am. Jour. Psychiat., 1923, iii, 111; Jour. Lab. Clin. Med., 1926, xi, 1187.
[2]Grossman: Jour. Ment. Sc., 1925, lxxi, 439.
[3]Harris: Brit. Med. Jour., 1926, No. 3395, p. 136.
[4]Aiello: Policlinico S. P., 1922, xxix, 537.
[5]Brugi: Pensiero Med., 1923, xii, 583.
[6]Fürth and Nobel: Biochem Ztschr., 1920, cix, 103; and Lieben, ibid, p. 124, 153.
[7]Hopkins and Cole: Jour. Physiol., 1902, xxvii, 418.
[8]Benedict, S. R.: Jour. Biol. Chem., 1909, vi, 51.
[9]Ling: Jour. Biol. Chem., lxix, 397.

ON THE CULTURAL CHARACTERISTICS OF LACTOBACILLUS ACIDOPHILUS*

By C. Roos,† Philadelphia, Pa.

As a starting point for this discussion, reference can be made to the work of Cruickshank and Berry,[1] on the presence of Bacillus acidophilus in human feces, reported in the British Medical Journal of November, 1924. The investigation referred to was made at the instance of the British Medical Council. The report dealt with the types of acidophilus bacilli found in fecal specimens from adults and children. As many as ten different strains were isolated from a single specimen. No relation between morphology, colony formation, sugar or milk reactions could be established, and the conclusion was reached that the term, L. acidophilus, covered a considerable group of organisms.

These conclusions of Cruickshank and Berry are generally in agreement with the reports of the earlier observers. Moro,[2] in 1900, described the L. acidophilus surface colonies as having a delicate hair-like periphery, denser towards the center, the deeper lying colonies showing the characteristic branching threads. Frequently one can see colonies of other forms without threads.

Some of our recent contributors have taken a very limited view in regard to the diagnostic cultural characteristics of L. acidophilus. Rettger[3] and his coworkers are inclined to regard as L. acidophilus only those strains that form L. bulgaricus-like colonies on agar, ferment certain carbohydrates and produce only a mild acidity in milk. A typical acidophilus colony, accordingly, on casein digest galactose agar, is one with fuzzy edges (when observed under the low power objective), and has been designated as Type x, and one showing only a very few hair-like projections designated as Type y. They would consider all other strains as unclassified intermediates. Kulp[4] has recently reaffirmed the earlier conclusions reached by Rettger and himself, and defines the typical L. acidophilus as a Gram-positive, nonmotile rod, which produces a mild acidity of 0.7 to 0.8 per cent in sterile milk employing twenty-four hours incubation at 37° C.

An acid culture of this degree and the flavor are of importance in the production of a palatable acidophilus milk, but the acid production is hardly suitable as a basis for classification, since the acidity produced varies with the amount of inoculum and the vigor of the strain. Moreover, the freshly isolated strains from the intestinal tract show the greatest variations in acid production in milk. Milk is coagulated by some strains in two days or even less; others may not coagulate milk at all. Coagulation generally is in pro-

*Read at the Annual Meeting of the Society of American Bacteriologists, Philadelphia, Pa., December 28, 1926.

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portion to growth rapidity and vigor of strain, regardless of the type of colony formed.

The colony most commonly met with in fecal specimens is of the solid type. The cells forming the solid colonies are far more resistant to deleteri-

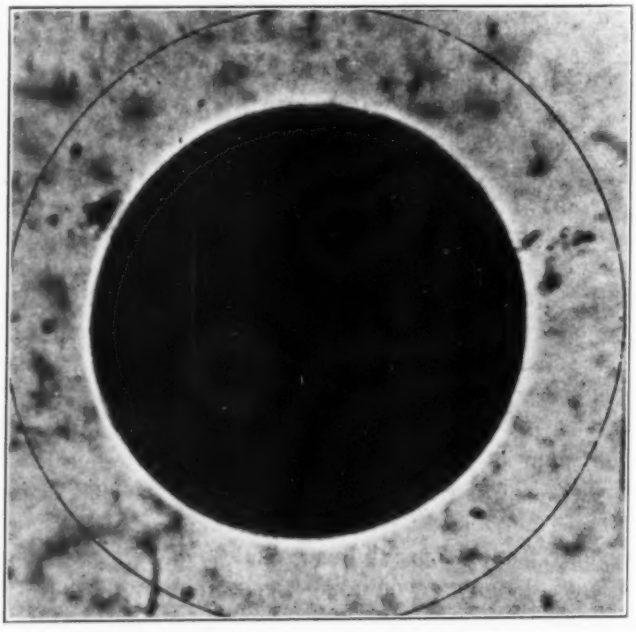

Fig. 1.—L. acidophilus, variant 1. Surface colony, two days old.

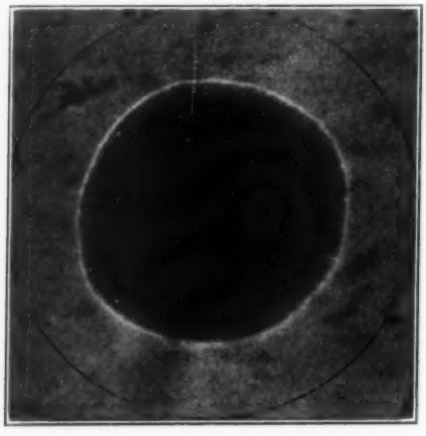

Fig. 2.—L. acidophilus, variant 2. Surface colony, two days old.

ous influences, both inside and outside the intestinal tract. They never disappear completely from the intestines, and on milk or carbohydrate diet they increase greatly in numbers. In some individuals the strains that produce solid colonies predominate persistently over all other types.

The filamentous type is most commonly obtained from fecal specimens

from individuals on milk or high carbohydrate diet. When individuals, showing no filamentous type colonies, are placed on milk diet or fed lactose over long periods the filamentous type colonies appear. Whence do they come?

Fig. 3.—L. acidophilus, variant 3. Surface colony, sixteen hours old.

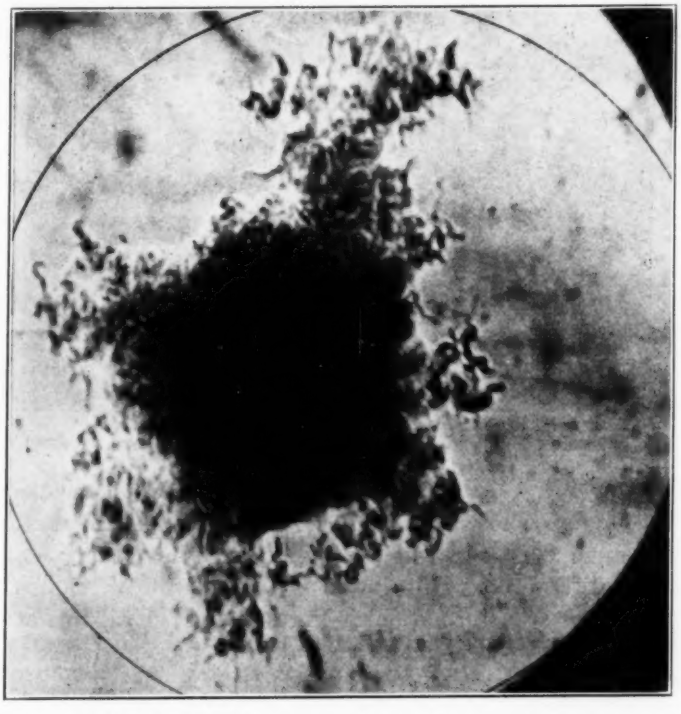

Fig. 4.—L. acidophilus, variant 3. Surface colony, twenty-four hours old.

Do they evolve from the solid type of those within as a result of the diet, or are they introduced from outside?

Our cultural findings, using acid-milk trypsin-digest agar media, of the acidophilic bacilli in human intestines have given us results comparable to

those reported by Cruickshank and Berry. On the basis of colony formation we have isolated as many as five distinct strains from one specimen. These strains all grew on media of such acidity (P_H 5.0) as would inhibit the growth of other organisms except some yeasts and cocci.

Our interest in L. bulgaricus and L. acidophilus preparations for therapeutic purposes dates back to 1913, and during this period, there have come under our observation many strains of L. acidophilus from authentic sources. All, except two, form L. bulgaricus-like colonies. One of these two has proved to be an especially vigorous strain, and is less fastidious in regard to its cultural requirements. This strain was isolated by us four years ago, from an acidophilus milk. Upon repeated platings on whey agar, the surface colonies were uniformly round and convex, enterococcus-like. The deep colonies were

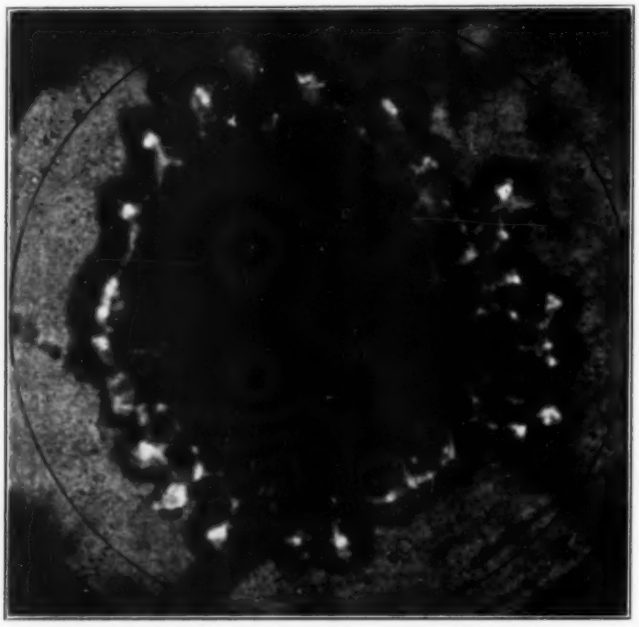

Fig. 5.—L. acidophilus, variant 3. Surface colony, five days old.

mostly beech-nut-like. Some were elliptical. In liquid media typical L. acidophilus growth occurred. A finely flocculent sediment formed with growth adhering to the sides, leaving the supernatant clear. Stained smears showed Gram-positive rods, uniform in size, single or in chains.

For purposes of transformation of the intestinal flora, this strain proved highly efficacious, but on account of its cultural characteristics some authorities regard it as atypical.

We consulted three of the seven members constituting, at present, the Committee on Lactic-Acid Ferment Preparations for the American Council on Pharmacy and Chemistry. Our first consultant informed us that it would not be practical to admit this strain as true L. acidophilus on account of the atypical colony. The second consultant stated that he had never seen an acidophilus like it, and doubted its authenticity.

The third consultant after a brief examination of our culture pronounced it as impure, consisting of three different strains. Upon closer examination he found that these strains were variants. Furthermore, he found that these variants differed in their power of carbohydrate fermentation. Fermentation tests made in duplicate gave the following results: Variant (1) negative to maltose, sucrose, unheated levulose; variant (2) positive to maltose, weakly positive to sucrose, negative to unheated levulose; variant (3) negative to maltose, and sucrose, weakly positive to unheated levulose. Surface tension tests showed that this strain, in common with other L. acidophilus strains, grows at 39 dynes, whereas L. bulgaricus does not grow below 42 dynes.

This culture of L. acidophilus has been carried along in sterile milk since its isolation four years ago. From this the following distinct types of colonies have been obtained through repeated selections: (1) an enterococcus-like colony, round, dense and smooth, Fig. 1; (2) streptococcus-like col-

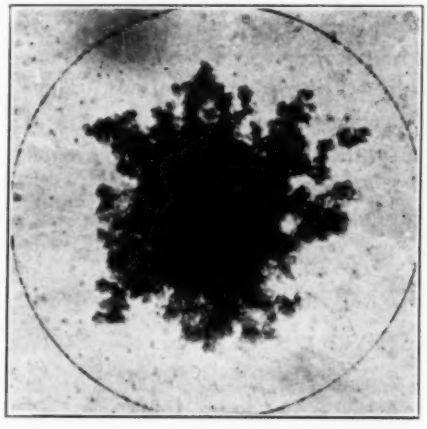

Fig. 6.—L. acidophilus, variant 3. Deep colony, five days old.

ony, the edges somewhat irregular, surface elevation raised and slightly umbonate, Fig. 2; (3) L. bulgaricus-like colony, Figs. 3, 4, 5 and 6. This latter colony, when about fifteen hours old, does not differ from L. bulgaricus or L. acidophilus Type x colony. All these colonies, when grown on milk trypsin-digest medium, remain true to type. Single colony fishings of any of these types placed in milk tend to produce cell types, some of which form the filamentous colonies when plated.

The filamentous colonies contain many longer rods, which show more tendency towards pleomorphism. The explanation seems tenable that the shorter rods of uniform size growing in palisade formation or in tortuous chains evolve solid or slightly irregular colonies, whereas the longer rods result in hair-like projections, such as is characteristic of L. bulgaricus. The evolution of these colony types can be readily observed under the microscope on casein-digest agar plates. Our observations are not confined to the above strain. Similar occurrences have been noted in cultures of other types. One of these cultures is a single cell isolation.

CONCLUSIONS

From our observations we conclude that the name L. acidolphilus should be applied to a group of biologically related strains, variable in cultural and morphologic characteristics.

We regard the various types of strains of lactobacilli found in the intestinal tract as variants.

Acid production, carbohydrate fermentation, colony formation are variable characteristics and alone do not constitute basis for classification. Nor do any of these characteristics serve as reliable indications of therapeutic potency. From the standpoint of clinical usage, source of the strain, its nutrient and physical requirements to facilitate growth, and temperature range are important factors and must necessarily be considered.

We believe that the presence of acidophilic bacilli in the intestinal tract of man is natural and beneficial. Culturally identical strains may show distinct immunologic differences, depending on human or animal sources, indicating a change due to adaptation to a particular environment. Therefore, it seems logical to use for therapeutic purposes only strains of L. acidophilus isolated from normal human sources.

Such a strain should be vigorous, not fastidious as to nutrient requirements, little sensitive to oxygen tension, capable of growth in a medium of varied surface tension, and with a wide temperature range. Such is the strain of choice, provided it meets the crucial test of proved therapeutic value.

Grateful acknowledgement is herewith made to Dr. John Reichel for helpful criticism and important suggestions.

REFERENCES

[1]Cruickshank, J., and Berry, D. W.: Brit. Med. Jour., Nov. 22, 1922, No. 3334, p. 944.
[2]Moro, Ernst: Jahrb. f. Kinderh., 1900, 38.
[3]Kulp, Walter L., and Rettger, Leo. F.: Jour. Bacteriol., July, 1924, ix, 357.
[4]Kulp, Walter L.: Jour. Am. Med. Assn., Sept. 11, 1926, lxxxvii, 833.

ACETONE BODY FORMATION AND THE CHEMICAL AFFINITY OF OXYGEN FOR CARBOHYDRATE AND FATTY ACID*

By Dwight M. Ervin, M.D., San Francisco, Calif.

IN A previous article it was shown mathematically that the blood-sugar curve and the excretion of glucose in the urine of a diabetic could not be explained upon the basis of failure to oxidize the glucose.[1] The purpose of this paper is an explanation of the chemistry of the "acetone bodies" found in the same pathology.

Fats and carbohydrates combine with oxygen in the work of the body. The body is a closed system of oxidation. Work is done (energy change) in the oxidation of each by a given quantity of oxygen held within a narrow limit of constancy.

Let W_1 = work done in oxidizing the fatty acid.
Let W_2 = work done in oxidizing the lactic acid.

Hence the ratio of oxygen taken by each is the ratio of the work done in the oxidation of each.

If a = quantity of oxygen liberated from hemoglobin
x = oxygen to fatty acid
a − x = oxygen to lactic acid.

$$\text{Hence } \frac{x}{a-x} = \frac{W_1}{W_2} = \frac{\text{chemical affinity of } O_2 \text{ for fatty acid}}{\text{chemical affinity of } O_2 \text{ for lactic acid}}$$

$$\frac{x}{a-x} = \frac{R\ T \log K_1 - R\ T\ \Sigma\ v \log C_1}{R\ T \log K_2 - R\ T\ \Sigma\ v \log C_2}$$

K^2 = Equilibrium constant of oxygen and lactic acid.†
K^1 = Equilibrium constant of oxygen and fatty acid.†

The distribution of oxygen between the fatty acid and lactic acid is determined by their chemical affinities.

It is the intention of this work to examine the above equation for an explanation of the formation of the "acetone bodies" that will be free of the necessity of the oxidation of glucose or one of its derivatives; free of that expression, "fats burn only in the fire of carbohydrates."

As recovery of the contracted muscle takes place energy equal to that given up on contraction must be returned to the muscle, and this energy is supplied by the oxidation of the fatty acids and carbohydrates (or its derivative). The "foods" are delivered by some mechanism that determines their ratio, to a given quantity of oxygen released from the hemoglobin system. The quantity of oxygen released is held within narrow limits to a constant quantity. The equation above will give the distribution of this oxygen between the carbohydrate and the fatty acid to be burned.

The quantity of work done by the carbohydrate (to be referred to as lactic acid for this is the probable form in which it is oxidized) and by the

*Received for publication, January 2, 1927.
†These equilibrium constants are now being determined.

fatty acid in restoring the muscle from a state of dissipated energy to its initial state will depend upon what part of the oxygen released from the hemoglobin each receives. From the equation above by keeping the oxygen quantity constant and altering the ratio of fatty acid to the lactic acid intermediate products of the fatty acid will be produced including the "acetone bodies." To this distribution the ratio of their respective affinities for the oxygen applies.

$W_1 = R\ T \log K_1 - R\ T\ \Sigma\ v \log C_1$
$W_2 = R\ T \log K_2 - R\ T\ \Sigma\ v \log C_2$
C_1 = equal the initial concentration of fatty acid reactants and oxygen.
C_2 = equal the initial concentration of lactic acid reactants and oxygen.

$$\frac{W_1}{W_2} = \frac{R\ T \log K_1 - R\ T\ \Sigma\ v \log C_1}{R\ T \log K_2 - R\ T\ \Sigma\ v \log C_2}$$

Since in the mixture the CO_2, acetic acid, and oxygen are common to both this may be written—

$$\frac{e^{\frac{W_1}{RT}}}{e^{\frac{W_2}{RT}}} = \frac{\frac{K_1}{C_1}}{\frac{K_2}{C_2}}$$

Cancelling the reactant and resultants common to both, the equation becomes—

$$\frac{e^{\frac{W_1}{RT}}}{e^{\frac{W_2}{RT}}} = \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid}}$$

$$\frac{W_1 - W_2}{R\ T} = \log \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid.}}$$

$W_1 = Q_1 a$ where Q_1 is an operator that converts the grams of oxygen used into calories when the oxygen is combining with the fatty acid; and

Q_2 = that which converts the oxygen into calories when combining with lactic acid.*

$W_2 = Q_2\ (a - x)$ for lactic acid combustion.

Substituting the equation becomes in terms of oxygen distribution instead of work terms—

$$Q_1\ x - Q_2\ (a - x) = R\ T \log \frac{K_1 \times \text{concentration of fatty acid}}{K_2 \times \text{concentration of lactic acid}}$$

From this equation it is seen that the affinity of each substance sharing the oxygen is measured by the equilibrium constant and the concentration. If the oxygen quantity be maintained constant and insufficient to completely oxidize both there will be formed intermediate products of the substance highest in concentration.

This equation is tested in the following manner for the explanation of the "acetone bodies."

The oxidizing agent used was potassium dichromate and hydrochloric acid. This mixture with either lactic or B-hydroxy butyric acid would come to an equilibrium of carbon dioxide pressure within twenty-four hours.

The method of analysis of B-hydroxy butyric acid and acetone (aceto

*From the work of F. G Benedict and E. L. Fox in Jour. Biol. Chem., lxvi, 783, both Q_1 and Q_2 will have the same value (10.8 per m. mol of oxygen for the small calorie).

acetic acid considered as acetone) was that of Van Slyke's with certain precautions to be discussed later.[2]

The method of oxidation was in the cold over a period of twenty-four hours. Both Shaffer[3] and Van Slyke[4] state that in the cold the oxidation of B-hydroxy butyric acid proceeded in some other way than through acetone. This from the figures of Van Slyke seems apparent unless another solution for Van Slyke's figures can be offered.[5]

It is possible that the oxidation of B-hydroxy butyric acid proceeds in both heat and cold through acetone, but in the method of Van Slyke the acetone as formed is caught in the insoluble compound—mercury sulphate—and removed from the field of oxidation, while in the cold the acetone is oxidized through to acetic and formic acid.

TABLE I

	$K_2Cr_2O_7$	HCl	BOH	ACETONE YIELD
24 hr. in cold	250 mg.	850 mg.	80 mg.	0.6 mg.
Heated in reflux 1½ hr. with dichromate and acid. $HgSO_4$ added	250 mg.	850 mg.	80 mg.	0.5 mg.
Dichromate added after boiling in $HgSO_4$ had begun	250 mg.	850 mg.	80 mg.	22.0 mg.

TABLE II*

	$K_2Cr_2O_7$	HCl	BOH†	ACETONE YIELD
24 hr. in cold	250 mg.		80 mg.	13.5 mg.
Heated in reflux 1½ hr. with dichromate and acid. Then $HgSO_4$ solution added	250 mg.		80 mg.	14.0 mg.
Dichromate added after boiling in $HgSO_4$ had begun	250 mg.		80 mg.	33.1 mg.

*The sulphuric acid dichromate mixture of Van Slyke's was then tried in experiment II.
†Kahlbaum (d-1) B-hydroxy butyric acid.

These two experiments were good evidence that both in the heat and cold the oxidation of B-hydroxy butyric acid proceeded through acetone, but in the technic of Van Slyke 0.75 mol acetone per mol of B-hydroxy butyric acid was caught in the insoluble compound and removed from the oxidation field while 0.25 of acetone slipped through to acetic and formic acid because of the speed of the reaction.

Van Slyke found that when he increased his initial reactants, chromate and sulphuric acid, he did not recover 0.75 mol per mol of the fatty acid.[6] When he increased his reactant he increased the speed of the reaction and more acetone would pass through to acetic and formic acid.

By increasing the acids the speed of the reaction is increased and a smaller yield of acetone will result unless the acid be the end product of the fatty acid oxidation. We may decrease the driving force of a reaction by adding one of the final derivatives of oxidized acetone. By adding acetic acid to the oxidation of B-hydroxy butyric acid in Van Slyke's technic we may slow the reaction of acetone to acetic acid and increase the yield of acetone if it is a question of the 0.25 mol slipping by the insoluble mercury compound; but not if the direction is some other than through acetone.

TABLE III

O	BoH	HCl	ACETIC ACID	ACETONE	YIELD PER MOL
73 mg.	80 mg.	0 mg.	0 mg.	33.5 mg.	0.75 mg.
73 mg.	80 mg.	0 mg.	200 mg.	40.5 mg.	0.90 mg.
73 mg.	80 mg.	850 mg.	0 mg.	22.2 mg.	0.50 mg.

TABLE IV

O	BoH	HCl	ACETIC ACID	ACETONE	YIELD PER MOL
73 mg.	75 mg.	0 mg.	0 mg.	30.4 mg.	0.74 mg.
73 mg.	75 mg.	0 mg.	200 mg.	37.5 mg.	0.91 mg.
73 mg.	75 mg.	850 mg.	0 mg.	21.8 mg.	0.49 mg.

Outside of the acetic acid added to the B-hydroxy butyric acid before placing it in the reflux the technic was identical to Van Slyke's.*

The presence of acetic acid, which is not oxidized by the dichromate mixture, was sufficient to retard the reaction to give a yield of 0.90 mol of acetone per mol of B-hydroxy butyric acid.

This action of acetic acid puts beyond doubt that the oxidation of the fatty acids proceeds through acetone in both heat and cold.

All other acids decrease the yield. Sulphuric acid gives a yield of 0.75 mol while the hydrochloric acid mixture yields 0.50 mol. Throughout the following experiment where the B-hydroxy butyric acid is computed from acetone when oxidized by the hydrochloric acid it is computed as one mol of the fatty acid to 0.5 mol of acetone.

TABLE V

OXYGEN	BoH	H_2SO_4	HCl	ACETONE	YIELD
73 mg.	80 mg.	Van Slyke	0 mg.	33.5 mg.	0.75 mol
73 mg.	80 mg.		850 mg.	22.2 mg.	0.50 mol
73 mg.	80 mg.		850 mg.	21.8 mg.	0.49 mol

The technic of the following experiments is: The required amounts of B-hydroxy butyric acid with potassium dichromate to yield 73 mg. of oxygen and 825 mg. of hydrochloric acid were placed in a 50 c.c. flask and made up to 15 c.c. in final volume. The flasks were set aside to oxidize for twenty-four hours at room temperature. At the end of this time except in the low concentrations of B-hydroxy butyric acid the clear green of the chromic acid was distinct showing that the oxidation was complete. The mixture of B-hydroxy butyric acid, lactic acid, acetone, and other derivatives was then placed in a 500 c.c. flask with 290 c.c. of Van Slyke's mercury sulphate acid mixture and boiled for one and one-half hours under a reflux. To this reflux was always attached a condensing tube which dipped below 30 c.c. of cold water. At the end of one-half hour this water was placed through the reflux condenser into the boiling flask. This was taken as a precaution to prevent any possible loss of acetone. At the end of one and one-half hours the solution was cooled and filtered through an alundum crucible. Since no other dichromate was added during this boiling the quantity of acetone formed in the distribution of the initial concentration of oxygen between lactic acid

*Acetic acid does not yield a precipitate with Van Slyke's reagents.

and B-hydroxy butyric acid would be given. The crucible was placed in a drying oven at 110° C. for one hour.

The filtrate was returned to the reflux apparatus and when brought again to boiling 10 c.c. of 5 per cent dichromate was added. When 320 mg. B-hydroxy butyric acid was used 15 c.c. of 5 per cent dichromate solution was used. At the end of one and one-half hours boiling the solution was filtered through a crucible and dried in the drying oven. This gave the B-hydroxy butyric acid not oxidized by the initial 73 mg. From these experiments could be deduced the quantity of oxygen taken by the fatty acid.

If the oxygen remained insufficient to completely oxidize the mixture of lactic acid and B-hydroxy butyric acid, then as the concentration of the B-hydroxy butyric acid increases from Van't Hoff's isotherm it can be reasoned that more oxygen will be taken by the B-hydroxy butyric acid in total but less per mol, which will cause more B-hydroxy butyric acid to be attacked by the oxygen but less completely burned.

TABLE VI

INCREASE OF B-HYDROXY BUTYRIC ACID

O	BOH	LACTIC	ACETONE	BOH RECOVERED	TOTAL O TO BOH	O PER MOL BOH	BOH OXIDIZED COMPLETELY
73 mg.	40 mg.	45 mg.	0.0 mg.	0.0 mg.	24.6 mg.	63.9 mg.	40.0 mg.
73 mg.	53 mg.	45 mg.	4.5 mg.	1.79 mg.	27.7 mg.	54.8 mg.	43.2 mg.
73 mg.	80 mg.	45 mg.	5.5 mg.	17.9 mg.	32.9 mg.	42.5 mg.	51.3 mg.
73 mg.	120 mg.	45 mg.	10.0 mg.	45.8 mg.	37.0 mg.	32.0 mg.	58.3 mg.
73 mg.	160 mg.	45 mg.	14.5 mg.	71.6 mg.	42.5 mg.	27.6 mg.	62.5 mg.
73 mg.	320 mg.	45 mg.	25.0 mg.	182.5 mg.	64.5 mg.	23.9 mg.	93.8 mg.
73 mg.	160 mg.	0 mg.	2.2 mg.	35.8 mg.	73.0 mg.		119.3 mg.

TABLE VII

O	BOH	LACTIC	ACETONE	BOH RECOVERED	TOTAL O TO BOH	O PER MOL BOH	BOH OXIDIZED COMPLETELY
73 mg.	80 mg.	0 mg.	0.5 mg.	0.0 mg.			
73 mg.	80 mg.	45 mg.	5.5 mg.	5.5 mg.	12.0 mg.		
73 mg.	80 mg.	180 mg.	7.0 mg.	23.9 mg.	2.0 mg.		
73 mg.	80 mg.						
73 mg.	160 mg.	0 mg.	2.2 mg.	35.8 mg.	73.0 mg.		119.3 mg.
73 mg.	160 mg.	45 mg.	14.5 mg.	71.6 mg.	42.5 mg.		62.5 mg.
73 mg.	160 mg.	155 mg.	15.5 mg.	124.7 mg.*	10.6 mg.		10.6 mg.
73 mg.	160 mg.	280 mg.	17.5 mg.	*			

*Large quantities of lactic acid yield a precipitate with Van Slyke's reagents.

With a mixture of lactic acid and B-hydroxy butyric acid to be oxidized by a constant quantity of oxygen we may by increasing the concentration of the B-hydroxy butyric acid theoretically produce a system in which all the B-hydroxy butyric acid has been oxidized to acetone. But as the acetone is formed a new distribution takes place. The oxygen is now distributed between the two acids and acetone.

Table VI shows the distribution of oxygen in such a system. Chiefly that as the B-hydroxy butyric acid is increased more oxygen is taken by the B-hydroxy butyric acid, but less per mol with a consequent increase of acetone.

In a similar manner there is an increase of acetone when the concentration of lactic acid is increased. Here, however, the total quantity of oxygen taken by the B-hydroxy butyric acid as well as the oxygen per mol is decreased.

It is interesting to note in the passing that when B-hydroxy butyric acid is only slightly above the quantity completely oxidized by a given quantity of oxygen, the ratio $\frac{\text{Acetone}}{\text{B-hydroxy butyric (unburned)}}$ became greater than one, but as the acid increases in concentration the ratio drops rapidly. This is to be expected from the equation. When the B-hydroxy butyric acid increases to a quantity where all the oxygen taken by the acid is less than mol for mol the B-hydroxy butyric acid will rise to about the acetone output.

Hubbard and Wright[7] noticed this discrepancy and ascribed it to the fact that there was a differential secretion of the kidney. This seems rather impossible when we find the same changing ratio in the blood.[8]

From Hubbard and Wright[8] and Joslin[9] are added some tables showing the ratio of acetone to the B-hydroxy butyric acid.

TABLE VIII

ACETONE	B-HYDROXY BUTYRIC
0.25 mg.	0.17 mg.
0.59 mg.	0.53 mg.
0.89 mg.	1.35 mg.
1.44 mg.	4.27 mg.
2.17 mg.	5.27 mg.

TABLE IX*

DAY	ACETONE	B-HYDROXY BUTYRIC
1	0.31 mg.	0.0 mg.
2	1.50 mg.	2.90 mg.
3	1.67 mg.	17.94 mg.
4	3.54 mg.	18.47 mg.
15	0.0 mg.	0.0 mg.
16	0.02 mg.	0.0 mg.
17	1.20 mg.	0.17 mg.
18	1.52 mg.	5.44 mg.
19	1.55 mg.	13.54 mg.

*Again from Joslin Table 133.

SUMMARY

An explanation of the "acetone bodies" formation based upon the theory that work done in the recovery of the system is obtained by the oxidation of fatty acids and glucose or its derivative.

With the theory that work is done by the oxidation of these two the distribution of oxygen between the two is then developed by Van't Hoff's isotherm, or equation for chemical affinity.

This chemical affinity of oxygen for fatty acid and lactic acid is a function of their equilibrium point and their concentration. The distribution of oxygen in oxidizing lactic acid and fatty acid is dependent upon the concentration of the lactic acid and the fatty acid.

When the concentration of oxygen is maintained constant and the concentration of the fatty acid increased to the degree that there is insufficient oxygen to completely oxidize the fatty acid there will result; the greater quantity of oxygen will be distributed to the fatty acid (decreased R Q) but a decreased quantity of oxygen per mol of fatty acid (increased intermediate derivatives of oxidation, acetone bodies).

The ratio $\frac{\text{acetone}}{\text{B-hydroxy butyric}}$ is a linear equation of the quantity of oxygen, the quantity of B-hydroxy butyric acid and the chemical affinity of oxygen for the two.

The ratio when small quantities are excreted in the urine is greater than one, when large quantities are excreted drops rapidly below one. This condition is paralleled in the above equation in the test tube.

The explanation offered for the chemistry of the "acetone bodies" is independent of the theory of the oxidation of glucose (lactic acid) and is applicable to all pathologic states where the quantity of fatty acid to be oxidized rises above a given ratio of oxygen per mol of fatty acid.

REFERENCES

[1]Ervin, D. M.: Jour. Lab. and Clin. Med., 1927, xii, 318.
[2]Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxxii, 455.
[3]Shaffer, P. A.: Jour. Biol. Chem., 1908-09, v, 211.
[4]Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxxii, 473.
[5]Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxxii, 473.
[6]Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxxii, 468.
[7]Hubbard and Wright: Jour. Biol. Chem., 1922, l, 400.
[8]Hubbard and Wright: Jour. Biol. Chem., 1921, xlix, 383.
[9]Joslin: New York, 1923, p. 308.

SPONTANEOUS DEATH IN A RABBIT WITH THE ISOLATION OF A TYPE IV PNEUMOCOCCUS*

By Everett S. Sanderson, Ph.D., Charlottesville, Va.

WHILE the small laboratory animals are susceptible to injections of pneumococci, rarely do these organisms seem to play a rôle in natural infections. Keegan[1] found them as secondary invaders in an epidemic among mice and guinea pigs caused by B. bronchisepticus, but the present isolated case is of interest in that an organism of this type was apparently the cause of spontaneous death.

The animal in question was from laboratory stock and in November had been injected intravenously and intraperitoneally with an old stock culture of anthrax for demonstration to students. Insofar as outward appearances were concerned, however, the injections had no ill effects, and about the middle of December the rabbit was housed with a normal one and the two kept in the main laboratory. The former animal was quite thin, but always

*From the Department of Pathology and Bacteriology, University of Virginia.
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active and eager for food, and at no time was there any indication of ill health. On the morning of January 27 it was found dead, the belief being that it had suddenly succumbed to latent anthrax. That this was not the case is evident from the following observations made at autopsy.

The peritoneal cavity contained a small amount of slightly reddish fluid, while the pericardial sac was filled with a clear serous fluid. The intestines and stomach were distended with gas, the spleen was not enlarged, and the gall bladder contained a yellowish, granular pasty material. The lungs were pale and not enlarged. Smears from the heart's blood showed vast numbers of Gram-positive, lancet-shaped diplococci possessing a distinct halo. A similar picture was observed in a smear from the liver. The pericardial fluid contained smaller numbers of diplococci and was free from cellular elements. A few macrophages were to be observed in the peritoneal fluid and the diplococci were numerous although less so than in the heart's blood. The organisms were extra cellular. In all these smears none but lancet diplococci were seen. Smears from the material in the gall bladder, however, showed great numbers of Gram-negative coli-form bacilli. The lancet diplococcus was isolated in pure culture from the spleen and peritoneal fluid, while in the heart's blood and pericardial fluid it was mixed with a colon bacillus of the type producing acid and gas from saccharose. This latter organism was obtained in pure culture from the gall bladder. No anthrax bacilli were found.

Five-tenths c.c. of a bouillon culture from the spleen injected intraperitoneally into a mouse caused death in twenty-four hours. The organisms in the exudate were numerous, soluble in ox-bile but failed to agglutinate with standard type sera. They were recovered from the heart's blood. Culturally the organisms produced acid and clot in inulin serum water and the colonies on blood agar were umbilical with formation of a slight amount of green pigment. These characteristics would indicate the organism to be a Type IV pneumococcus.

The finding of innumerable pneumococci in the smears of the heart's blood of this case, together with similar organisms in pericardial and peritoneal fluids, would lead one to suggest that the animal had succumbed to a pneumococcus septicemia. As was mentioned above the two rabbits had been housed in a room daily occupied by students and so it might be reasonable to assume that the infection was air borne. To ascertain whether the companion rabbit was also harboring this organism, cultures were made from the anterior nares and pharynx. Pneumococci were not recovered and a cardiac blood culture remained sterile after eight days' incubation.

SUMMARY AND CONCLUSIONS

A spontaneous death in a stock rabbit with isolation of a Type IV pneumococcus from the heart's blood, spleen, peritoneal and pericardial fluids has been described. Microscopic pictures from smears of heart's blood and tissues would indicate a pneumococcus septicemia.

REFERENCE

[1]Keegan, J. J.: Arch. Int. Med., 1920, xxvi, 570.

A STUDY OF BILE SECRETION FROM A CASE OF BILIARY FISTULA*

By S. Gordon Ross, M.B. (Tor.), Pasadena, Calif.

THE bile secretion in a case with biliary fistula was studied to determine the amount and uniformity of the daily secretion and the effect thereon of ingested bile and various diets. This study was made in conjunction with Dr. Harry S. McGee.

CASE REPORT

D. V. R., male, aged 56 years, height 70 inches, weight 110 pounds, was admitted to the Pasadena Hospital August 14, 1925, in a deeply jaundiced and cachectic condition. He gave a history of two operations. The first was for excision of a gastric ulcer in 1918,

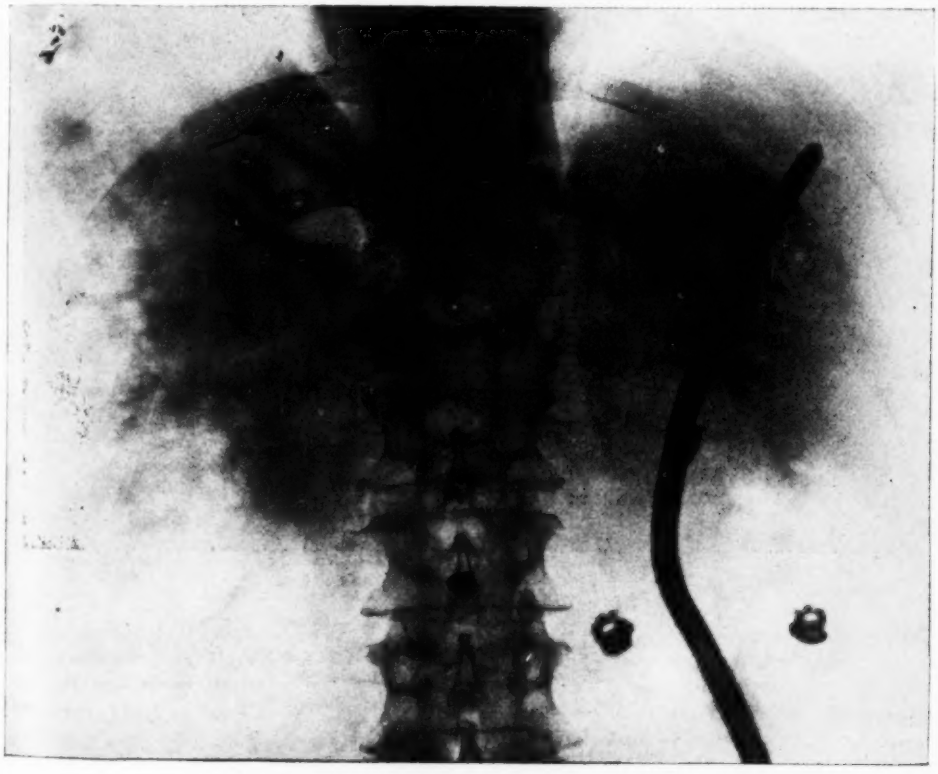

Fig. 1.—Immediately before injection of lipiodol.

necessitated by severe hemorrhage. In November, 1924, an acute intestinal obstruction was relieved by loosening adhesions around the upper small intestine. Jaundice developed after this operation and became progressively deeper until his skin was deeply bronzed. At opera-

*From Metabolic Department, Pasadena Hospital.
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tion on August 24, 1925, for this condition, the abdominal cavity was found to contain a great many adhesions in the upper right quadrant. The common bile duct was so enlarged as to resemble the small bowel. On opening it, a thorough exploration revealed no stones, either in it or in the hepatic ducts. The Ampulla of Vater was found embedded in a mass of dense fibrous tissue. A fine probe could not be introduced into the duodenum, through the Ampulla. A catheter was inserted in the duct as a drain. The gall bladder could not be found.

The wound healed tightly around the catheter allowing no loss of bile. The patient developed a desire to drink his own bile which he took, as required, from a bottle held by a belt around his waist.

Our studies on this case started in April, 1926. At this time the jaundice had completely disappeared. He weighed 140 lbs. and was able to carry on light work. His admission into the hospital at this time was solely for the purpose of this study, as he otherwise was apparently normal.

Fig. 2.—Immediately after injecting lipiodol.

In studying biliary fistulae, it is of the utmost importance to rule out the presence of a functioning gall bladder. In addition to the evidence obtained from the bile itself, we have an operative report that no gall bladder was found. The biliary tract was further investigated by injecting lipiodol into the drainage tube with the patient in Trendelenberg's position. X-ray plates were made by Dr. C. H. Parker, before injection, just after injection and three days after injection. No gall bladder outline could be seen, although the biliary radicles were very well outlined.

Daily Amount.—The twenty-four hour bile secretion varied from 725 to 1010 c.c., the average being 863 c.c. The average twelve-hour day specimen contained 523 c.c. and the corresponding night specimen contained 340 c.c. These figures were obtained during a period when he ingested about 700 c.c. of bile daily. On discontinuing the bile ingestion, the 24-hour secretion decreased to an average of 556 c.c. ranging from 505 to 725 c.c. This is shown clearly in the accompanying graph, as well as in Table I.

TABLE I

BILE INTAKE C.C. PER 24 HOURS	BILE SECRETION				
	DAY	NIGHT	TOTAL DAY		
	8 A.M.-8 P.M.	8 P.M.-8 A.M.	LOW	HIGH	AVERAGE
Average 700 c.c.	523	340	725	1010	863
No intake	360	225	505	725	556

Daily Uniformity of Secretion.—The daily specimen was collected at two-hour intervals in order to determine if daily maxima occurred in the secretion. It was found that, for two-hour periods during the day, the amounts were remarkably uniform:

8 A.M.-10 A.M.	85 c.c. bile
10 A.M.-12 M.	83 " "
12 M.- 2 P.M.	86 " "
2 P.M.- 4 P.M.	88 " "
4 P.M.- 6 P.M.	93 " "
6 P.M.- 8 P.M.	83 " "

Fig. 3.—Three days after injecting lipiodol.

Specific Gravity.—During bile ingestion the specific gravity varied between 1.008 and 1.012. When no bile was taken, the specific gravity dropped to 1.006 and 1.010. This is in accordance with the finding of Wisner and Whipple.[1]

The fact that the bile was continually about 1.010 specific gravity suggests strongly that we were dealing with bile secreted directly from the liver, and uninfluenced by the concentrating function of the gall bladder. Gall bladder bile and biliary fistula bile are differentiated in Hawk.[2] The former usually has a specific gravity of about 1.040.

Diet.—During the first ten days of the sixty-day period of observation, a regular hospital diet was given: Fat, 90 gm.; protein, 95 gm.; carbohydrate, 280 gm.; calories, 2310. The average daily bile secretion was 860 c.c. A lower caloric diet was used then with the protein increased: carbohydrate, 86; protein, 134; fat, 96; calories, 1744. The secretion on this diet diminished to 780 c.c. It was poorly tolerated by the patient. It appeared that when he complained of the food, the bile secretion diminished. His intolerance for fats made it impossible to study a high fat diet. Carbohydrates, especially fruit juices and glucose, were tolerated well by the patient. A diet of carbohydrate, 450 gm.; protein 40 gm.; fat, 20 gm.; calories, 2140, was given. This diet was very acceptable to him, and resulted in an average twenty-four hour secretion of 900 c.c.

Blood Chemistry.—During a high carbohydrate diet that was well tolerated, the blood chemistry showed:

Nonprotein nitrogen	35.1 mg. per 100 c.c. blood
Uric acid	2.1 mg. per 100 c.c. blood
Creatinine	2.0 mg. per 100 c.c. blood
Sugar	100.0 mg. per 100 c.c. blood
Chlorides	430.0 mg. per 100 c.c. blood
CO_2 Vol. per cent	80 per cent

These readings are within normal limits.

During a high protein diet, the blood chemistry showed:

Nonprotein nitrogen	46.2 mg. per 100 c.c. blood
Uric acid	4.7 mg. per 100 c.c. blood
Sugar	154.0 mg. per 100 c.c. blood
Chlorides	460.0 mg. per 100 c.c. blood
CO_2 Vol. per cent	45 per cent

which we interpret as a moderate retention of protein catabolites accompanied by moderate acidosis.

Special interest was taken in studying the blood calcium and phosphorus, because of the changes noticed in blood clotting time in jaundiced cases, and the osteoporosis that sometimes results in cases of complete biliary fistula of long standing. The blood calcium and phosphorus were determined several times during the periods of bile ingestion as well as the period when no bile was ingested, and they were found to remain constant,—calcium, 12.0 mg. per 100 c.c. blood, and phosphorous, 4.5 mg. per 100 c.c. blood.

SUMMARY

1. Bile secretion varied from 725 c.c. to 1010 c.c. per twenty-four hours during bile ingestion.

2. Bile secretion varied from 505 c.c. to 725 c.c. per twenty-four hours during no bile ingestion.

3. Bile was secreted at a constant rate during the day. The secretion tended to diminish during the night.

4. The quantity and specific gravity of the bile were both increased by bile ingestion.

5. High carbohydrate diet was best tolerated. High fat diet could not be tolerated. High protein diet resulted in nonprotein nitrogen retention, accompanied by moderate acidosis.

6. Blood calcium and phosphorus remained constant and independent of bile ingestion.

7. Lipiodol may be used to investigate a biliary fistula and the biliary radicles.

REFERENCES

[1]Wisner, F. P., and Whipple, G. H.: Am. Jour. Physiol., March, 1922, lx, No. 1.
[2]Hawk, Philip B.: Practical Physiological Chemistry, ed. 8, Philadelphia, 1923, page 206.

BARBITURIC ACID DERIVATIVES AS ANESTHETICS AND METHODS FOR ADMINISTRATION*

By J. Frank Pearcy and M. M. Weaver

IN 1922 Tatum and Parsons[1] described in this journal the use of barbital as an anesthetic for dogs. Since that time other derivatives of barbituric acid have been studied as hypnotics and have been placed upon the market for their clinical use. Having found barbital very useful in our experiments on secretion and bulbospinal reflexes[2] we thought it profitable to study the newer preparations as anesthetics for animals.

Tatum and Parsons described only the per os administration of barbital-sodium for anesthesia in animals. This has been the method in general use. Although it is the most physiologic method of administration, other methods are practically much superior. Believing that the little use which is being made of this valuable anesthetic is due to unfamiliarity with easy and certain methods of administration, we think it timely to describe them in some detail

Per Os Method.—The ordinary stomach tube method is used. It is well to provide that the animal shall not have been fed the day it is to be anesthetized because of uncertainty of absorption and danger of vomiting. If for a minute or two following withdrawal of the stomach tube the attendant holds the animal's head up and administers a friendly pat or two, the possibility of vomiting is made negligible, that is, at least if one has not introduced too much water into the stomach.

This method of administration, although the most physiologic, has serious

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drawbacks. If any of the solution is vomited the animal usually has to be discarded for several days, as one cannot readily replace the lost drug with any accuracy. The time required for surgical anesthesia is variable due to variations in gastrointestinal motility and absorption. The animal may be ready in three-quarters of an hour, but it may require three or four hours. The degree of anesthesia is also variable, presumably due to failure of part of the drug to be absorbed.

Intramuscular Method.—This is the method of choice. Administration is easy, anesthesia is rapidly produced, and variations in the time required for the production of anesthesia and in the depth of the anesthesia are reduced to a minimum. The solution is injected deep into the gluteal muscles, one-half into each side. The administration is quickly and easily performed. It possesses none of the disadvantages of the previous method. The animal is under surgical anesthesia in from twenty to forty minutes.

Intraperitoneal Method.—This is a simple and fast method. Satisfactory anesthesia is obtained in from fifteen to thirty minutes. It has the disadvantage, however, of producing edema and hyperemia of the omentum and mesentery, and a considerable amount of fluid collects in the peritoneal cavity.

Intravenous Method.—This is, of course, the most rapid method. Surgical anesthesia is obtained in from five to twenty minutes. The injection must be made slowly to prevent the development of shock. "Shock" can usually be prevented by injecting divided doses, one-third at a time, and five to ten minutes apart.

The method has the advantage of being rapid. If divided doses are given, however, it is hardly faster than the intramuscular method. It is more difficult to administer the drug in this way as the animal must be carefully and steadily held while the saphenous vein is pierced and the injection slowly made or else it must be done under ether anesthesia.

The dosage varies with the method of administration, the age, and the size of the animal. The basic dose of barbital-sodium given per os is 0.3 gm. per kg. The basic dose given intramuscularly or intraperitoneally is 0.27 gm. per kg. Intravenously the dose is 0.20 to 0.25 gm. per kg. Young and old dogs require smaller dosage. Small animals must be given from 0.1 to 0.3 gm. per kg. additional whereas for large dogs the dose is decreased by from 0.1 to 0.5 gm. per kg. Cats require a smaller dosage, 0.25 gm. per kg. usually being adequate when given per os, although very small cats may require 0.3 gm. per kg. or even more.

If the barbitalized animal shows spontaneous movements it may be quieted by injecting more barbital or by injecting $\frac{1}{8}$ to $\frac{1}{4}$ gr. of morphine sulphate. After an animal has been under barbital anesthesia for several hours it may become restless, due to reflexes set up by a distended bladder or colon. In such cases the bladder or colon should be emptied.

If the animal is too "deep" under the anesthetic it can frequently be made suitable for experimental work by giving from $\frac{1}{200}$ gr. to $\frac{1}{100}$ gr. of eserine phosphate and from $\frac{1}{2}$ gr. to 1 gr. caffeine.

After proper anesthesia is obtained the animal should be permitted to

"sleep" (in a warm place) for a few hours. The reflexes become much more responsive.

Barbital is rather difficultly soluble. It may be neutralized by $NaHCO_3$, as recommended by Tatum and Parsons, but the use of the now commercially obtainable barbital-sodium is more satisfactory. Barbital-sodium is soluble 1 gm. in 6 c.c. of distilled water. The basic dose of barbital is 0.25 gm.; of barbital-sodium it is 0.3 gm.

Phenobarbital or *Luminal* is almost insoluble in water. It may be neutralized in N/2 NaOH. This sodium salt is approximately one-half as soluble as barbital sodium. The dose for dogs is from 0.12 gm. to 0.14 gm. per kg., or about one-half the barbital sodium dose. No advantage could be detected in its use. On the contrary, it is troublesome to prepare and is markedly more toxic.

Iso-amyl-ethyl-barbituric acid or *Amytal* is difficultly soluble in water. The sodium salt is more soluble than barbital-sodium but its preparation is tedious and the solution is unstable. The manufacturers recommend 0.05 gm. per kg. This dose may be increased to advantage.

Calcium-ethyl-isopropyl-barbiturate or *Ipral* is quite difficultly soluble in water. Its use is limited, therefore, to per os administration. The disadvantages of this method have been pointed out above. A dosage of 0.15 gm. per kg. is suitable.

Thus these substances all have disadvantages not possessed by barbital-sodium. Excepting those cases where survival is desired these derivatives have no advantages over barbital-sodium as an anesthetic for most experimental studies such as those upon secretion and reflex action.

SUMMARY

1. The details are given for administering barbital-sodium to animals for anesthesia per os, intramuscularly, intraperitoneally, and intravenously.

2. The advantages and disadvantages of these methods are pointed out.

3. Barbital-sodium is more suitable for anesthesia than barbital.

4. Luminal, Amytal and Ipral have disadvantages but no advantages over barbital-sodium for anesthesia. Suitable dosages are recorded.

We wish to express our thanks to Professor Carlson for many invaluable suggestions.

REFERENCES

[1]Tatum and Parsons: Jour. Lab. Clin. Med., 1922-23, viii, 64.
[2]Pearcy and Weaver: Am. Jour. Physiol.

THE DEMONSTRATION OF BACTERIOPHAGE IN OLD STOCK CULTURES*

By Gordon M. Kline, Albany, N. Y.

NUMEROUS attempts have been made to demonstrate the presence of bacteriophage in so-called "normal" cultures. Bail,[1, 2] in 1921, succeeded in isolating bacteriophage from old broth cultures of three strains of *B. dysenteriae* Flexner, and later from nine strains of *B. dysenteriae* Shiga by exhausting the broth medium with repeated inoculation and centrifugalization. Otto, Munter, and Winkler[3, 4] and Weinberg and Aznar[5, 6] followed this early report with similar observations of bacteriophage obtained from stock strains. Later investigations by many workers, including Seiffert,[7] Jotten,[8] Monteiro,[9] Pondman,[10] Gildemeister and Herzberg,[11] Flu,[12, 13] Reichert,[14] Burgers and Bachmann,[15] and Kuttner[16] and Mallmann[17] in this country, failed to reveal the presence of bacteriophage in laboratory bacterial strains except in a very few exceptional instances. With regard to those cultures from which bacteriophage was isolated, many of the investigators agree with Twort[18] who, in his original article describing the lytic phenomenon, reported that the lytic substance might be produced spontaneously in bacterial cultures, whereas others believe with d'Herelle[19] that these cultures were contaminated with bacteriophage at the time of isolation.

The methods which were used most generally by previous workers in seeking to isolate bacteriophage from the cultures, were either a comparatively long period of incubation (ten days to six months) or a process based on the Buchner zymase extraction method—trituration with sand and subsequent extraction under pressure. The long-continued incubation, however, would be apt to obscure any bacteriophage that might have been present, for it is generally conceded that bacteria finally become resistant to the lytic action and may then destroy the bacteriophage. To quote from d'Herelle,[19] p. 197, "A bacteriophage, then, becomes attenuated during the same process which leads to an acquisition of resistance by the bacterium. Even a bacteriophage of maximum virulence may be 'overcome.'" Recently, Shwartzman[20] has shown that prolonged standing in the incubator weakens the lytic principle, even in the absence of bacteria. Since it may well be that the failure of trituration and extraction of the bacterial cell is due to the fact that the lytic substance is normally present in an inactive form, the following experimental studies were undertaken.

Experimental Work.—In investigating our stock cultures, it was decided to adopt the same technic that is used in testing stool filtrates for bacteriophage. All the media employed were adjusted to P_H 7.8.

*From the Division of Laboratories and Research New York State Department of Health. Presented in abstract before the American Association of Pathologists and Bacteriologists, April 14-15, 1927, Rochester, N. Y.

TABLE I

HISTORIES AND AGGLUTINATIVE ACTIVITY OF LYTIC CULTURES

CULTURE	HISTORY		AGGLUTINATION			
	SOURCE	DATE	1:200	1:1000	1:2000	1:5000
B. coli communis (186)	Am. Museum Natural History	1915				
B. typhosus (Bender 270C)	Bender Laboratory, Albany	1915	4+	3+	3+	+
B. dysenteriae Mt. Desert (114 E)	N. Y. C. Research Laboratory	1915	4+	4+	4+	3+
" " " (114 H)	Div. of Lab. and Research	1916	3+	4+	4+	4+
" " " (114 O)	Rockefeller Institute	1916	4+	4+	4+	3+
" " " (114 U)	"	1916	4+	4+	4+	2+
" " " (114 X)	College of Phys. and Surg.	1916	4+	4+	3+	+
" " " (114 O/1)	University of Oxford	1920	4+	4+	4+	3+
" " " (114 U/1)	Div. of Lab. and Research	1916	4+	4+	3+	2+
" " Shiga (114 F)	N. Y. C. Research Laboratory	1915	3+	3+	2+	+
" " " (114 V/1)	Pasteur Institute, Paris	1923	−	−	−	−
" " Flexner (114 D)	N. Y. C. Research Laboratory	1915	3+	3+	3+	2+
Staphylococcus aureus	Div. of Lab. and Research	1919				
" *albus*	" " " "	1919				

Method.—Agar slants were inoculated with the culture under investigation and incubated at 37° C. for from five to seven hours. The growth was washed down with 2 c.c. of sterile beef-infusion broth and from 0.05 to 0.2 c.c. of the suspension were inoculated into 10 c.c. of beef infusion broth to yield a slight turbidity; 0.05 c.c. of this latter suspension were spread on an agar plate. After from eighteen to twenty-four hours' incubation, the tube and plate were examined for evidences of lysis. If there was any indication of lysis, the contents of the tube were filtered through a Berkefeld candle and 0.5 c.c. were inoculated into a fresh suspension of the bacteria and the procedure repeated.

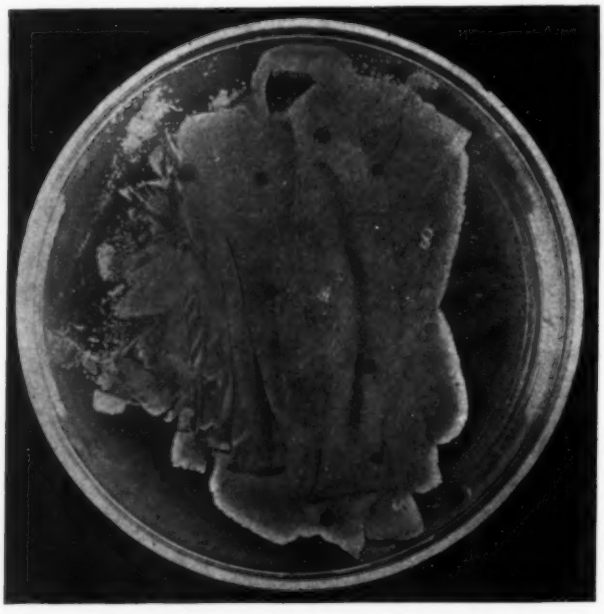

Fig. 1.—*B. dysenteriae* Mt. Desert No. 114 E. Lytic filtrate diluted 10^{-7}.

By this method, lytic substance was observed in 14 of 21 cultures studied (see Table I) distributed as follows:

Cultures Studied	*No. Found Lytic*
2 *B. coli*	1
3 *B. typhosus*	1
7 *B. dysenteriae* (Mt. Desert)	7
6 *B. dysenteriae* (Shiga)	2
1 *B. dysenteriae* (Flexner)	1
2 *Staphylococcus*	2

Of the cultures found to be lytic, bacteriophage was isolated from eleven, being identified by the characteristic transmissibility in series and formation of plaques. The other three cultures, namely, the Flexner strain and the two strains of staphylococci, gave such evidences of lysis as sterile plates spread

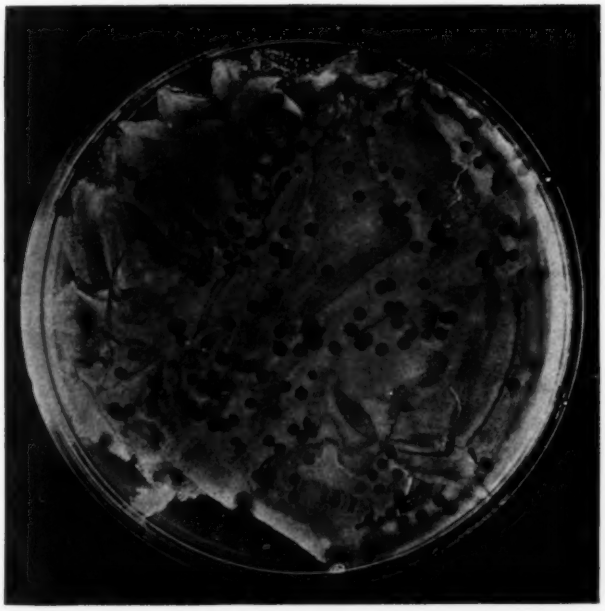

Fig. 2.—*B. dysenteriae* Mt. Desert No. 114 U. Lytic filtrate diluted 10^{-6}.

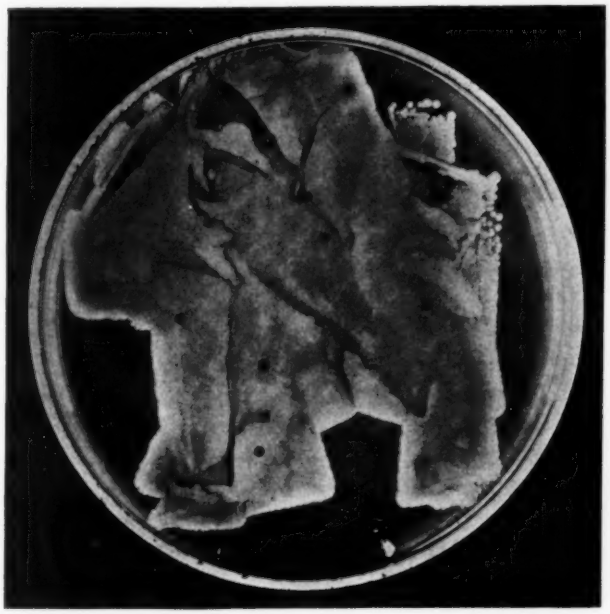

Fig. 3.—*B. dysenteriae* Mt. Desert No. 114 U/1, Control plate.

from slightly turbid suspensions, colonies becoming transparent and formation of plaques, but to date no success has followed attempts to obtain lysis in series.

The isolation of bacteriophage was not always successful on the first test; it was rather the exception to find the culture lytic at the start. Many times, neither the tubes nor the plates would show any signs of lysis. Sometimes, the tube would be clear and the plate show perfectly confluent growth; then again, the tube was very cloudy and the plate showed scant growth or plaques.* In many instances, duplicate tubes and plates, inoculated at the same time from the same bacterial suspension, gave these opposite extremes of growth, one tube and plate being sterile, whereas the other set showed normal growth. This variation in the lytic manifestations of the cultures is

Fig. 4.—*B. dysenteriae* Mt. Desert No. 114 U/1. Lytic filtrate diluted 10^{-6}.

very evident in the protocol included herein, giving observations of the tubes and plates inoculated with the bacteria only, over a period of a few months.

The lytic filtrates obtained from the cultures were subjected to dilution and plating in order to study the plaques so obtained. It was found that the seven strains of *B. dysenteriae* Mt. Desert when plated with their homologous filtrates, gave both a large type of plaque (3 to 3.5 mm. diameter), and a small type of plaque (about 1 mm. diameter); the *B. typhosus* strain also developed two sizes of plaques: one about 1 mm. in diameter, the other, a scarcely visible "pin-point" plaque. Only small plaques (about 1 mm. diameter) were ob-

*The latter phenomenon was sometimes found to be a case of proliferation of resistant organisms in the presence of high concentration of bacteriophage, as Gohs[21], and others, have observed; this was noticed very often, particularly in making dilutions of the bacteriophage for estimating its strength and to obtain individual plaques. It was found that usually after from eighteen to twenty-four hours' incubation, the 10^{-1} and 10^{-2} dilutions were very cloudy, while the others showed little growth or were perfectly sterile. By observing the tubes after from three to four hours' incubation, however, it was found that lysis had occurred in these higher concentrations.

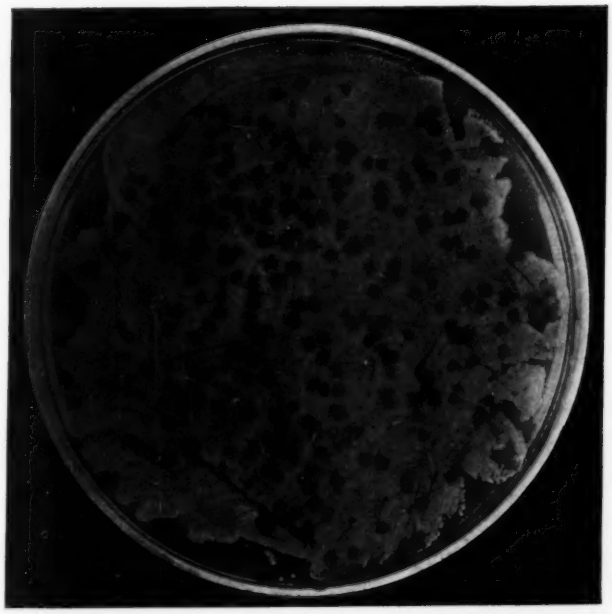

Fig. 5.—*B. dysenteriae* Mt. Desert No. 114 O. Lytic filtrate diluted 10^{-7}.

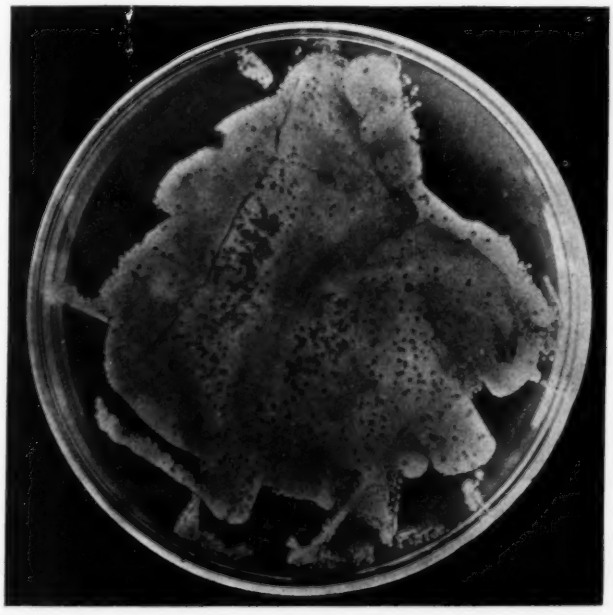

Fig. 6.—*B. dysenteriae* Mt. Desert No. 114 O/1. Lytic filtrate diluted 10^{-9}. (Note the one large plaque in the upper border of the growth.)

PROTOCOL 1

B. dysenteriae MT. DESERT (114 E_{10})

SPONTANEOUS APPEARANCE AND DISAPPEARANCE OF LYTIC ACTIVITY
(INCUBATION PERIOD TWENTY-FOUR HOURS)

DATE	TUBE	PLATE	DATE	TUBE	PLATE
1927			1927		
1/20	4+	4 transparent colonies	2/14	4+	Scattered growth
	–	Confluent growth	2/15	4+	Confluent growth
1/24	–	Confluent growth	2/18	3+	Confluent growth
1/25	4+	Many plaques	3/1	–	Confluent growth
	–	Confluent growth	3/9	3+	45 large plaques
	4+	Sterile			3 small plaques
1/26	–	Confluent growth	3/10	+	7 large plaques
2/1	–	Confluent growth	3/12	–	Confluent growth
2/2	4+	Many plaques	3/14	–	2 large plaques
	–	Confluent growth		–	Confluent growth
2/3	–	Confluent growth	3/15	–	70 small plaques
2/10	4+	4 large plaques			4 large plaques
			3/18	4+	100 small plaques
					12 large plaques

tained from the *B. coli* and the two Shiga strains under the same conditions (Figs. 1 to 12). These results seemed to indicate two distinct lytic mechanisms operating in some bacteria. In order to prove the independence of

Fig. 7.—*B. coli communis* No. 186. Lytic filtrate diluted 10^{-4}.

the two mechanisms, the large and small plaques from the Mt. Desert strains were fished and replated separately with the homologous strain. It was observed that the large plaques gave rise to only large plaques and the small plaques to small ones.* Moreover, the filtrates containing lysin developing

*This is true only when the strain itself is not at the lytic threshold, as will be shown later.

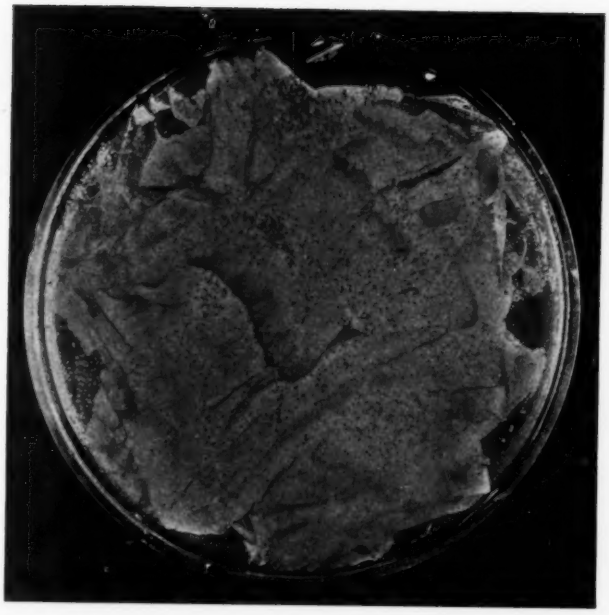

Fig. 8.—*B. typhosus* No. 270 C. Lytic filtrate diluted 10^{-3}.

Fig. 9.—*B. dysenteriae* Shiga No. 114 F. Lytic filtrate diluted 10^{-4}.

small plaques were active against some species on which the lysin, identified by the large plaques, had no effect. The separate entity of each of the two lytic mechanisms operating spontaneously in the Mt. Desert strains studied is, therefore, established. Furthermore, the identity of the large plaques and of the small plaques obtained from each of the seven Mt. Desert strains characterizes the lytic manifestations as inherited processes rather than due to chance contamination with foreign parasites.

One of the Mt. Desert strains used in this study, had, in the course of another experiment, been fished every six months from a single colony during the five-year period from 1920 to 1925, and the fishing used to continue the strain. D'Herelle has found that this procedure with cultures either artificially or naturally containing bacteriophage will eliminate the bacterio-

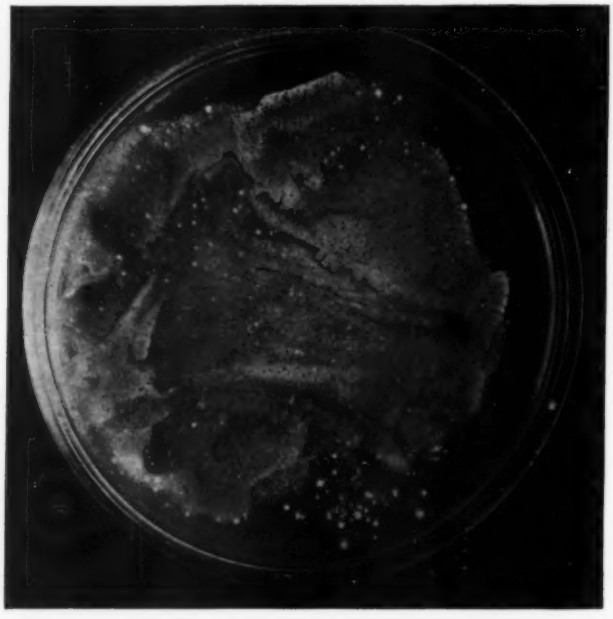

Fig. 10.—*B. dysenteriae* Shiga No. 114 V/1. Lytic filtrate diluted 10^{-4}.

phage from the strain except in very rare cases. It was thought possible, therefore, that the above culture would either be free of lytic substance entirely or that one of the bacteriophagic types (represented by either the large plaque or the small plaque) would have been removed. Examination of this culture, however, designated as 114 E_{10} showed that the ten colony isolations had eliminated neither the lytic properties of the culture as a whole nor either one of the lytic mechanisms active in the strain before colony isolation.

A study of the agglutinability of the cultures from which bacteriophage was isolated gave results contrary to the view commonly expressed in the literature with regard to the agglutination of lytic cultures (or cultures "contaminated" with bacteriophage). D'Herelle[19] states, on page 236, that, "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," and recently, Hadley[22] has confirmed this

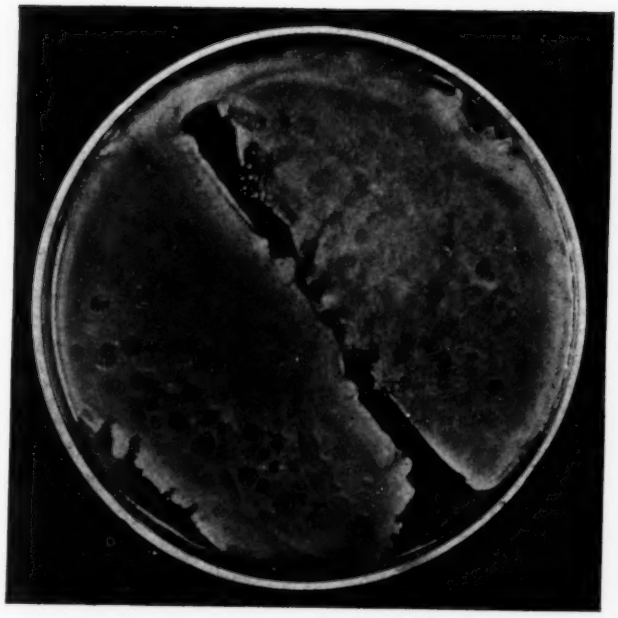

Fig. 11.—*Staphylococcus aureus* No. 58 B.

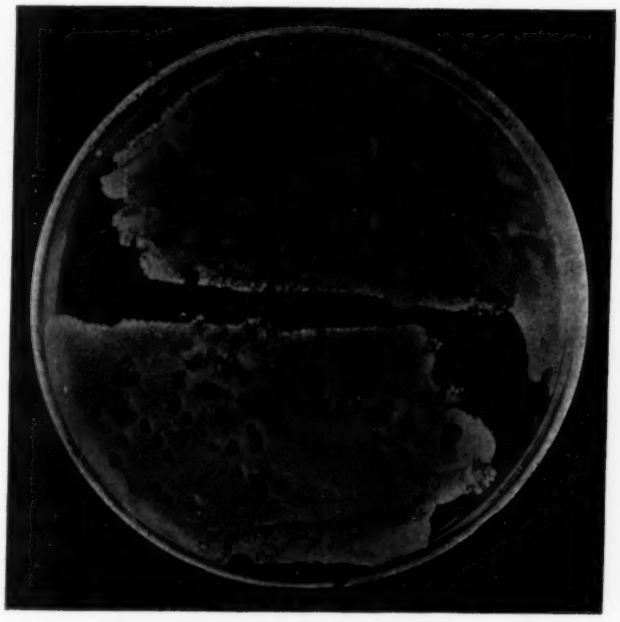

Fig. 12.—*Staphylococcus albus* No. 56 A.

statement. The agglutination of all our cultures, however, was normal (see Table I) with only one exception. The correlation of agglutinability with bacteriophagic phenomena may perhaps have been overemphasized. Bacteria freshly isolated from human infections are often practically inagglutinable. Metchinkoff and Bordet[23] found that bacterial strains showing normal agglutination became inagglutinable by remaining with leucocytes; Dawson[24] has reported considerable variation in the agglutinability of identical strains of bacteria grown in different media. Many similar instances of the effect of controlled environmental changes on the agglutination of bacteria have been demonstrated. In view of these observations, inagglutinability is not necessarily an accurate criterion of the presence of bacteriophage in bacterial strains.

DISCUSSION

It has been d'Herelle's contention that stock cultures in which bacteriophage has been found were contaminated by the ultravirus when originally isolated. The accumulation of observations, however, seems to indicate that the lytic state may occur spontaneously at a certain stage in the metabolism of the organisms. Bordet[25] and Lisbonne and Carrère[26] found that *B. coli* strains became lytic spontaneously and as quickly became "normal" again. Hadley[27] found that *B. pyocyaneus* suddenly became lytic after having grown normally through many generations. Bagger[28] in a recent study of the enterococcus, mentions that one of his strains "which previously had many times been inoculated in the same manner" suddenly gave rise to extensive bacteriophagous activity. The observations on the cultures recorded in this paper reveal not only spontaneous appearance and disappearance of bacteriophagic action in bacterial cultures, but also an extreme variation in the lytic activity of bacteria from the same agar slant inoculated in duplicate into broth medium and immediately spread on agar. In the latter instance, it was noted that one agar surface was completely sterile after twenty-four hours, while the other showed perfectly confluent growth. It is difficult to conceive that such a phenomenon depends on the chance circumstance that one agar surface had been spread with bacteria for the most part contaminated with a foreign parasite, whereas the bacteria spread on the other agar surface had been entirely free of the parasite. It is more likely that the phenomenon is related to other spontaneous dissociations, now widely recognized, occurring constantly among the various bacterial species.

The apparent hereditary character of the bacteriophage races obtained spontaneously from the lytic cultures is very striking. It is generally conceded that, although the size of the plaques may be made to vary by changing the environmental conditions, nevertheless, under the same conditions, a plaque, when fished and plated with fresh culture, will develop many more of the same size. This has been found to be true for both the large and the small Mt. Desert plaques. It must be thought, therefore, that a large plaque represents a definite lytic mechanism (B) as compared to that mechanism (b) developing small plaques. Since plaques of two different sizes appeared on the plates of the lytic Mt. Desert strains and of the lytic *B. typhosus*

strains, we have experimental evidence of the presence of at least two distinct lytic mechanisms in some bacterial cells. This presence of more than one bacteriophage race in a single strain readily explains the phenomenon observed by Seiffert[29] that the development of resistance to one lytic agent might be accompanied by a newly acquired sensitiveness to another. He, and later Burnet,[30] reasoned there must be more than one lytic ferment present, and this I have found to be the case.

Hadley,[31] in 1924, found that a lytic filtrate of the "small" strain only, acting on Shiga gave only small plaques whereas a lytic filtrate of the "large" strain gave both large and small plaques. He suggests that the exciting cause is susceptible of independent variation, and compares the phenomenon with his nonlytic and lytic pyocyaneus colonies, the first giving rise to nonlytic colonies only, the second to both lytic and nonlytic colonies. In my experiments in which the large and small plaques of the Mt. Desert culture were fished and replated separately, it was usually possible to obtain each one to the exclusion of the other, but occasionally one or more of the series would show a mixture of plaques. Inasmuch as the control plates also gave plaques, separately or mixed, spasmodically, the occasional appearance of both types on a plate to which only one type plaque was fished is evidence merely that the culture itself was approaching the lytic threshold when it would spontaneously produce bacteriophage, and that the stimulus applied to one lytic mechanism by the filtrate added was sufficient to discharge another also. Only small plaques were found in the two Shiga cultures giving bacteriophage spontaneously; this would seem to indicate that the mechanism (b) in the Shiga bacillus is easily set off, in Hadley's case, by the mere operation of another mechanism (B).

SUMMARY

Previous investigations of old stock cultures have demonstrated bacteriophage in only a small percentage of cases, possibly due to the methods employed. When the technic used in examining stool filtrates for bacteriophage was applied to stock cultures, lytic substance was observed in 14 of 21 cultures studied. Typical bacteriophage, as identified by transmissibility in series and formation of plaques, was obtained from 11 of the 14 lytic cultures: namely, 1 *B. coli*, 1 *B. typhosus*, 2 *B. dysenteriae* Shiga, and 7 *B. dysenteriae* Mt. Desert strains. Tubes and plates, inoculated with the bacteria only, over a period of a few months varied from complete lysis to "normal" growth. In some instances, duplicate tubes and plates, inoculated at the same time from the same bacterial suspensions, gave these opposite extremes. This spontaneous appearance and disappearance of bacteriophagic action in these cultures associates the lytic phenomenon with other spontaneous microbic variations. The 7 *B. dysenteriae* Mt. Desert strains gave both large plaques (3 to 3.5 mm. diameter) and small plaques (about 1 mm. diameter), indicating the presence of at least two distinct lytic mechanisms characterized as inherited processes rather than as chance contaminations with foreign parasites. One of the Mt. Desert strains was fished from isolated colonies ten times over a period of five years, but this procedure failed to eliminate either the lytic

property of the culture as a whole, or either one of the lytic mechanisms active in the strain before colony isolation. Although d'Herelle states that "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," the agglutinability of the cultures containing bacteriophage was normal with only one exception.

REFERENCES

[1]Bail, O.: Bakteriophage Wirkungen gegen Flexner und Koli-Bakterien, Wien. klin. Wchnschr., 1921, xxxiv, 448.

[2]Bail, O.: Über Shiga-Bakteriophagen, Wien. klin. Wchnschr., 1921, xxxiv, 555.

[3]Otto, R., and Munter, H.: Zum d'Hérelleschen Phänomen, Deutsche med. Wchnschr., 1921, xlvii, 1579.

[4]Otto, R., and Winkler, W. F.: Über die Natur des d'Hérelle'schen Bakteriophagen, Deutsche med. Wchnschr., 1922, xlviii, 383.

[5]Weinberg, M., and Aznar, P.: Autobactériolysines et le phénomène de d'Hérelle, C. r. Soc. de Biol., 1922, lxxxvi, 833.

[6]Weinberg, M., and Aznar, P.: Quelques faits nouveaux sur les autobactériolysines, C. r. Soc. de Biol., 1922, lxxxvii, 136.

[7]Seiffert, W.: Über das d'Hérelleschen Phänomen, Soziale Hyg. u. Mikrobiol., Sept. 21, 1922, Cited by d'Herelle.[19]

[8]Jötten, K. W.: Über das sogenannte d'Hérellesche Phänomen, Klin. Wchnschr., 1922, i, 2181.

[9]Monteiro, J. L.: Sobre o phenomeno de d'Hérelle. Presença do principio lytico nas culturas, em meio solido, do Bacillo anthracis, B. pestis e B. dys. Shiga-Kruse, Brazil Med., 1922, i, 297.

[10]Pondman, A.: Proeven tot het verkrijgen van het verschijnsel van d'Herelle uit reinculturen, Leiden C. de Boer. jr., 1923, Cited by d'Herelle.[19]

[11]Gildemeister, E., and Herzberg, K.: Über das d'Herellesche Phänomen. III. Mitteilung, Centralbl. f. Bakteriol., 1924, xci, 12.

[12]Flu, P. C.: Die Natur des Bakteriophagen und die Bildung von Bakteriophagen in alten Bouillonkulturen pathogener Mikroorganismen, Centralbl. f. Bakteriol., 1923, xc, 362.

[13]Flu, P. C.: Over Cholerabacteriophagen, Geneesk. Tijdschr. v. Vergelijk, 1924, x, 196, Cited by d'Herelle.[19]

[14]Reichert, F.: Untersuchungen über das d'Hérellsche Phänomen, Centralbl. f. Bakteriol., 1924, xci, 235.

[15]Bürgers, and Bachmann, W.: Bakteriophagen studien. I. Versuche mit grampositiven Mikroorganismen, Ztschr. f. Hyg. u. Infektionskrankh., 1924, ci, 350.

[16]Kuttner, A. G.: Bacteriophage Phenomena, Jour. Bact., 1923, viii, 49.

[17]Mallmann, W. L.: Old Stock Cultures As a Source of Bacteriophage, Jour. Bact., 1925, x, 59.

[18]Twort, F. W.: An Investigation on the Nature of Ultra-Microscopic Viruses, Lancet, 1915, clxxxix, 1241.

[19]d'Herelle, F.: The Bacteriophage and Its Behavior, Baltimore, Williams and Wilkins, 1926.

[20]Shwartzman, G.: Observations on Lytic Principle of Weak Potency, Proc. Soc. Exper. Biol. Med., 1924-25, xxii, 433.

[21]Gohs, W.: Die lytische Wirkung des Shiga bakteriophagen bei verschiedenen Konzentrationen, Wien. klin. Wchnschr., 1925, xxxviii, 481.

[22]Hadley, P.: Microbic Dissociation; the Instability of Bacterial Species with Special Reference to Active Dissociation and Transmissible Autolysis, Jour. Infect. Dis., 1927, xl, 1.

[23]Bordet, J.: Studies in Immunity, New York, John Wiley and Sons, 1909.

[24]Dawson, A. I.: Bacterial Variations Induced by Changes in the Composition of Culture Media, Jour. Bact., 1919, iv, 133.

[25]Bordet, J.: Apparition spontanée du pouvoir lysogène dans les cultures pures, C. r. Soc. de Biol., 1924, xc, 96.

[26]Lisbonne, M., and Carrère, L.: Sur l'apparition spontanée du pouvoir lysogène dans les cultures pures, C. r. Soc. de Biol., 1924, xc, 265.

[27]Hadley, P.: Transmissible Lysis of Bacillus Pyocyaneus, Jour. Infect. Dis., 1924, xxxiv, 260.

[28]Bagger, S. V.: The Enterococcus, Jour. Path. Bact., 1926, xxix, 225.

[29]Seiffert, W.: Der Charakter des d'Hérelleschen Phänomens, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1923, xxxviii, 292.

[30]Burnet, F. M.: The Nature of the Aequired Resistance to Bacteriophage Action, Jour. Path. Bact., 1925, xxviii, 407.

[31]Hadley, P.: The Variation in Size of Lytic Areas and Its Significance, Jour. Bact., 1924, ix, 397.

A COMPARISON OF 10,000 WASSERMANN AND KAHN TESTS RUN IN PARALLEL*

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SINCE the introduction of a flocculation test for the diagnosis of syphilis by Kahn[1] several years ago, several articles have appeared in the literature comparing the advantages or disadvantages of this test with those of the Wassermann reaction. A bibliography of the most important contributions is appended to this report.[2]

Although many articles have been published on this subject we feel justified in presenting the results of this series of tests because we believe that our figures portray a comparison of the two tests more accurately than the majority of the previous reports. Many have reported mere percentages of the agreement and disagreement between the two tests. Such figures are based on the total number of tests run and do not take into consideration the presence or absence of syphilis in the patients whose sera are tested. These reports have shown an approximate agreement between the two tests in 88 to 96 per cent of the tests run. In the remaining 4 to 12 per cent about one-half, or 2 to 6 per cent, of the tests have shown a positive Wassermann and a negative Kahn and a like number have shown a negative Wassermann and a positive Kahn. There have also been a number of reports in which a careful clinical analysis has been made. Such reports naturally give more accurate information regarding the two tests in the presence of syphilis but in these reports also the percentages are based on the total number of tests run.

Houghton, Hunter and Cajigas,[2] in a comprehensive report of a large series of tests, have presented the most convincing arguments for the adoption of the Kahn test in place of the Wassermann test. They conclude that the Kahn test is more sensitive than the Wassermann test in all stages of syphilis but more particularly so in cases of primary syphilis and in treated cases. In view of the many technical advantages of the Kahn test and its equal reliability with the Wassermann test they recommend the adoption of the Kahn test in place of the Wassermann test.

The other side of the question has been presented quite adequately by Kilduffe[2] who points out a number of real dangers and disadvantages of the Kahn test. He mentions the relative difficulty in securing a satisfactory Kahn antigen and the necessity of frequent titration of the antigen. He also emphasizes the importance of having the Kahn test conducted only by a thoroughly trained and competent technician.

TECHNIC

The following Wassermann technic is used in this laboratory. Two antigens are used, one being a simple alcoholic beef heart extract antigen, and

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the other being the same antigen to which cholesterol (0.6 per cent) has been added. A 5 per cent suspension of sheep cells is used and the hemolytic system is titrated before each test. The amount of serum used is 0.1 c.c. The tests with the cholesterolized antigen undergo a one-hour water-bath fixation while the tests with the alcoholic antigen are subjected to a four-hour ice-box fixation. We believe that such technic makes the two antigens about equally sensitive. The Kahn test is run strictly in accordance with the instructions given by Kahn.[1] The same cholesterolized antigen is used for both the Wassermann and Kahn tests and the Kahn tests are read after a period of fifteen to eighteen hours' incubation at 37.5° C.

TABLE I*

A COMPARISON OF 10,000 WASSERMANN AND KAHN TESTS RUN IN PARALLEL. THE PERCENTAGES BASED ON THE TOTAL NUMBER OF TESTS RUN

WASSERMAN AND KAHN AGREE THROUGHOUT			
++++	6.16%		
+++	0.16		
++	0.17		
+	0.13	6.62	
−		69.60	76.22
WASSERMANN AND KAHN AGREE QUALITATIVELY BUT DIFFER QUANTITATIVELY			
Kahn More Sensitive.—			
W.C. equals K. both stronger than W.A.	9.33		
W.A. equals K. both stronger than W.C.	0.19		
K. stronger than either antigen of W.	6.11	15.63	
Wassermann More Sensitive.—			
W.A. equals W.C. both stronger than K.	0.33		
W.C. stronger than either W.A. or K.	1.05		
W.A. stronger than either W.C. or K.	0.24	1.62	17.25
Wassermann and Kahn Show Complete Discrepancy.—			
K. pos. W. neg.	3.33		
W. pos. K. neg.	3.20		6.53
			100.00

*This table is similar to the many tables already in the literature and gives the percentages of agreement and disagreement between the two tests based on the total number of tests in the series. We found a complete quantitative agreement in 76.22 per cent of the tests. In 17.25 per cent of the tests there was a qualitative agreement but a quantitative difference. Of these differing quantitatively 15.61 per cent were in favor of the Kahn test and only 1.62 per cent in favor of the Wassermann test. Combining the two groups we found a qualitative agreement in 93.47 per cent of the tests run. In 6.53 per cent of the tests there was a complete discrepancy between the Wassermann and Kahn results. Of these, 3.33 per cent gave a negative Wassermann and a positive Kahn and 3.2 per cent gave a positive Wassermann and a negative Kahn.

TABLE II†

CLINICAL ANALYSIS OF TESTS SHOWING AN ABSOLUTE DISCREPANCY. 653 CASES

	UNDETERMINED	SYPHILITIC	NONSYPHILITIC	TOTAL
K. pos. W. neg.	9	261	63	333
W. pos. K. neg.	2	199	119	320
	11	460	182	653

†This table is an attempt to show the results of a clinical analysis of those cases which gave an absolute discrepancy between the Wassermann and Kahn Tests. We have designated as "syphilitic" those cases which either presented clinical syphilis or gave a definite history of having had a chancre or a previously frankly positive serology which had been altered by treatment. The cases designated as "nonsyphilitic" neither presented the clinical picture of syphilis nor gave a history suggesting syphilis and in many of these cases subsequent tests revealed a negative serology. We admit that such a division is not free from the possibility of error, but it does give as accurate information regarding the presence of syphilis as is possible to secure. Of the 653 cases coming under this group, there were eleven in which the clinical analysis was impossible so we have designated these cases as "undetermined."

DISCUSSION

The test which gives the more accurate information regarding the presence or absence of syphilis in the patient whose serum is tested is the test which will be adopted eventually. Figures based on the total number of tests run will vary with the number of negative tests in the series. For this reason percentages, to be of value, must be based on the reaction of the tests in a series of sera from syphilitic patients. Furthermore, a test which is so sensitive that it gives a reaction in the absence of syphilis is almost as objectionable as one which fails to reveal syphilis when present. It is our opinion that such procedures as a prolonged ice-box fixation may very well increase the sensitivity of the Wassermann reaction beyond the point of specificity. This danger is not so apparent with the Kahn test. The ability of the average practitioner to interpret, correctly, the results of the Wassermann test in

TABLE III*

COMPARISON OF THE WASSERMANN AND KAHN TESTS IN 2847 SYPHILITIC SERA

Total sera 2847.	K. Neg. in 199.	Failure of Kahn = 6.9 per cent.
Total sera 2847.	W. Neg. in 261.	Failure of Wass. = 9.1 per cent.

COMPARISON OF WASSERMANN AND KAHN TESTS WITH REGARD TO "FALSE POSITIVES"

Percentages based on number of known false reactions (Table II) and total number of positive reactions.

Pos. Kahn reactions 2720.	False pos. 63.	Falsely pos. 2.3 per cent.
Pos. Wass. reactions 2707.	False pos. 119.	Falsely pos. 4.4 per cent.

*This table shows a comparison of the two tests with sera from syphilitic patients. We are assuming that the 662 sera (Table I) where we found quantitative agreement with positive reactions are all from syphilitic patients. We also assume that the 1725 sera (Table I) which showed only a qualitative agreement but again all positive reactions, are from syphilitic patients. We know from analysis that the 460 sera (Table II) of the complete discrepancy group are from syphilitic patients. The sum of these groups make a total of 2847 syphilitic sera.

Such a comparison demonstrates without a doubt that neither test is infallible and that the error with either test is actually much greater than other reports have indicated. The Kahn test, however, is definitely more dependable where there is the question of diagnosis. The table also indicates that each test will give an appreciable number of false positive reactions, but here also the Kahn test appears to be definitely more reliable.

sera which gives only a partial reaction is variable. The laboratory man who conducts the tests is able, no doubt, to interpret his results according to the technic which he has employed, but it must be remembered that the general practitioner may attach an entirely different significance to the reaction. A glance through the literature will readily reveal the fact that some laboratory men consider a one- or two-plus reaction, with only one antigen, in the Wassermann test as a positive and significant test, while other men consider such a reaction as a negative test. A partial reaction with the Kahn test is more uniformly interpreted as being significant.

As has been pointed out by Kilduffe[2] the Kahn test is surrounded by far too many pitfalls to be safely employed as an office procedure by the general practitioner. A source of error which has not been emphasized in the literature is the comparative thermolability of the Kahn reaction. This fact makes the inactivation of the serum a procedure which must be conducted most carefully and at the lowest temperature possible.

It will be noted that in the 3.2 per cent of the tests (Table I) where the Kahn apparently failed, syphilis was present in only 1.99 per cent and two of

the cases were "undetermined." This indicated that the Kahn actually failed in less than 2 per cent of the tests run. Reasoning in a similar manner, in the 3.33 per cent where the Wassermann apparently failed, syphilis was present in 2.6 per cent with nine cases "undetermined." So the Wassermann failed in at least 2.6 per cent of the total number of tests run.

TABLE IV

ANALYSIS OF ABSOLUTE DISCREPANCIES—653 CASES = 6.53 PER CENT OF TESTS RUN

	K.	W.C.	W.A.	NUMBER OF CASES		TOTAL	KNOWN FALSE REACTIONS		TOTAL
K. pos. W. neg.	++++	−	−	8			1		
	+++	−	−	30			4		
	++	−	−	235			43		
	+	−	−	60		333	15		63
W. pos. K. neg.	−	++++	++++	10			4		
W.C. equals W.A.	−	+++	+++	5			1		
	−	++	++	7			2		
	−	+	+	28	50		10	17	
W.C. stronger	−	++++	−	6			1		
than W.A.	−	+++	++	3			0		
	−	+++	+	7			1		
	−	+++	−	8			2		
	−	++	+	14			3		
	−	++	−	52			18		
	−	+	−	136	226		63	88	
W.A. stronger	−	++	++++	6			3		
than W.C.	−	+	++++	7			3		
	−	−	++++	8			3		
	−	++	+++	3			0		
	−	+	+++	4			0		
	−	−	+++	5			1		
	−	+	++	8			3		
	−	−	++	2			1		
	−	−	+	1	44	320	0	14	119
						653			182

Key to Tables.
K = Kahn
W = Wassermann
W. A. = Wassermann, alcoholic
W. C. = Wassermann, cholesterol
Neg. = Negative
Pos. = Positive

This table gives a more detailed picture of the group of cases represented by Table II. In those sera which showed a complete discrepancy the degree of reaction between the two tests is shown. The degree of the "false positive" tests is also shown.

It is significant to note that the complete discrepancies between the two tests occurred for the most part in sera reacting weakly with either one or the other test. The "false positive" reactions with both tests also occurred for the most part in weakly reacting sera.

CONCLUSIONS

1. The Kahn test fails to detect syphilis in 6.9 per cent of cases.

2. The Wassermann test fails to detect syphilis in 9.1 per cent of cases.

3. In the diagnosis of syphilis the Wassermann and Kahn tests should preferably be run in parallel.

4. The Kahn test is more specific than the Wassermann test. (At least when compared with the Wassermann technic as used in this laboratory, and that technic is sensitive enough to give reactions beyond the point of specificity.)

REFERENCES

[1]Kahn, R. L.: The Kahn Precipitation Test for Syphilis, Am. Jour. Pub. Health, 1924, xiv, 498.

Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation: Governing Principles, Procedure and Clinical Application of the Kahn Precipitation Test, Baltimore, Williams & Wilkins Co., 1925.

[2]Anderson, C. M.: Value of Parallel Kolmer and Kahn Tests as Standardized Laboratory procedure in Serum Diagnosis of Syphilis, Canadian Med. Assn. Jour., 1926, xvi, 1211.

Becker, S. W.: Comparison of the Kahn Precipitation Test and the Kolmer Complement Fixation Test, with Consideration of the Difficulties in the Diagnosis of So-Called Latent Syphilis, Am. Jour. Syph., 1927, xi, 131.

Duemling, W. W.: Clinical Observations on the Kahn Test, Jour. Ind. Med. Assn., July, 1926, xix, 272.

Giordana, A. S.: Kahn Precipitation Test as Compared with Kolmer Complement-Fixation Test, JOUR. LAB. AND CLIN. MED., Feb., 1926, xi, 435.

Greenbaum, S. S.: Error of Basing Serum Diagnosis of Syphilis on the Kahn Reaction Alone, Jour. Am. Med. Assn., 1926, lxxxvi, 1273.

Hopkins, J. G., and Brunet, W. M.: The Kahn Precipitation Test for Syphilis, Jour. Am. Med. Assn., 1927, lxxxviii, 311.

Houghton, J. E., Hunter, O. B., and Cajigas, T.: The Kahn Test, Jour. Am. Med. Assn., 1926, lxxxvii, 1898.

Kelly, R. L.: Clinical Study of Kahn Precipitation Test and Kolmer Complement-Fixation Test, Arch. Derm. Syph., Nov., 1925, xii, 720; JOUR. LAB. CLIN. MED., Feb., 1926, xi, 437.

Kilduffe, Robt. A: Wassermann vs. Flocculation Tests in the Serological Study of Syphilis, Suitability of Kahn Test as an Office Procedure, Arch. Path. and Lab. Med., 1926, ii, 866.

Lederer, A: Comparative Value of Wassermann and Kahn Tests, U. S. Vet. Bur. Med. Bull., May, 1926, ii, 456.

Litterer, W.: Comparison of the Wassermann Test with the Kahn Test in 17,000 Serums, Jour. Tenn. St. Med. Assn., Sept., 1926, xix.

McGlumphy, C. B.: Flocculation Test for Syphilis, Arch. Path. Lab. Med., 1926, ii, 709.

Owen, R. G., and Cope, H. E.: Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, JOUR. LAB. CLIN. MED., Feb., 1926, xi, 432.

Redfield, K. T.: The Kahn Test as an Aid in the Diagnosis of Syphilis, Am. Jour. Syph., 1926, ix, 354.

LABORATORY METHODS

THE DETERMINATION OF FIBRIN IN BLOOD PLASMA*

By Joseph Chandler, Ph.D., Boston, Mass.

IN the fall of 1925 I undertook a series of chemical studies of the blood during pregnancy. Among the constituents of which the concentration was to be determined, was fibrin. While the concentration of fibrin in blood has been determined for a long time, it is only within a comparatively few years that methods which offer a reasonable degree of speed, accuracy and economy of material have been available. Some of the earlier methods have been described by Meek,[1] who as late as 1912 determined fibrin by whipping a measured quantity of whole blood, grinding, washing with water and sodium chloride solution until the wash liquor was biuret free, filtering off the fibrin, and, after washing with alcohol and ether, drying and weighing directly.

Within the past five years several excellent methods have been published. In most cases oxalate or citrate plasma has been used, which, after dilution with sodium chloride solution, is recalcified, allowed to clot, the fibrin removed, washed and either dried and weighed or the nitrogen determined by some modification of the Kjeldahl method. A review of twelve typical methods is given by Starlinger.[2] Cullen and Van Slyke,[3] and Howe[4] use the Kjeldahl method, while Gram,[5] and Foster and Whipple[6] prefer the gravimetric method. The refractometric method has been applied to the determination of fibrin in blood by Winternitz,[7] Leendertz and Gromelski,[8] and Leendertz.[9] An earlier method in which the smallest volume of magnesium sulphate plasma which will coagulate when mixed with a definite volume of fresh serum, is that proposed by Wohlgemuth.[10]

Wu[11] has recently proposed a very ingenious procedure. One c.c. of oxalate plasma is diluted with 28 c.c. of 0.8 per cent sodium chloride solution, recalcified with 1 c.c. of 2.5 per cent calcium chloride solution and the mixture allowed to clot for twenty minutes; the clot is loosened by gentle shaking and poured on a dry filter. The fibrin is removed by winding on a fine glass rod, dried with filter paper and placed in a 15 c.c. graduated centrifuge tube. Four c.c. of 1 per cent sodium hydroxide solution are added and the mixture heated in a boiling water bath until the fibrin is disintegrated. When cool, 10 c.c. of water are added and mixed thoroughly and the mixture centrifuged. The clear supernatant liquid is poured into a 25 c.c. volumetric flask, 1 c.c. of 5 per cent sulphuric acid, 0.5 c.e. phenol reagent and 3 c.c. of 20 per cent sodium carbonate solution are added, and after making up to volume and letting stand for fifteen minutes, the color is compared with that of a standard tyrosine solution.

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Wu's method seemed to offer the three advantages of accuracy, rapidity and economy of material, and was adopted tentatively for the investigation. The results obtained, however, were not entirely satisfactory. There was a strong tendency for a white precipitate to form during the fifteen minutes' interval of standing prior to the color comparison. The color also seemed to vary somewhat with the time of heating with the sodium hydroxide, and this difficulty could not be avoided easily as the heating must be continued until the fibrin is completely disintegrated.

Finally this method was modified with satisfactory results. The method of isolation of the fibrin suggested by Wu was retained, but for his colorimetric method, the determination of the nitrogen in the precipitated fibrin by a modification of the Folin microkjeldahl method was substituted. Results from duplicate determinations on the same plasma sample gave good agreement as is shown in Table I.

TABLE I

AGREEMENT OF DUPLICATE DETERMINATIONS

NO.	NAME	PER CENT PLASMA FIBRIN			CLASSIFICATION
		I	II	Mean	
1	R. M.	0.271%	0.277%	0.274%	Normal
2	A. G.	0.263%	0.263%	0.263%	Normal
3	E. T.	0.399%	0.377%	0.388%	Normal
4	L. H.	0.254%	0.243%	0.249%	Normal
5	E. Ry.	0.256%	0.253%	0.255%	Normal
6	E. Ru.	0.234%	0.217%	0.226%	Normal
7	F. K.	0.313%	0.313%	0.313%	Normal
8	L. S.	0.237%	0.243%	0.240%	Normal
9	L. S.	0.128%	0.122%	0.125%	Menstruation
10	L. S.	0.233%	0.241%	0.237%	Normal
11	L. S.	0.211%	0.206%	0.208%	Menstruation
12	E. W.	0.274%	0.277%	0.276%	Normal
13	E. W.	0.233%	0.235%	0.234%	Menstruation
14	R. P.	0.308%	0.300%	0.304%	Menstruation
15	G. C.	0.377%	0.352%	0.365%	Pregnancy, normal
16	I. R.	0.389%	0.404%	0.396%	Pregnancy, normal
17	M. R.	0.468%	0.451%	0.459%	Pregnancy, normal
18	E. C.	0.490%	0.490%	0.490%	Pregnancy, normal
19	B. B.	0.435%	0.438%	0.437%	Pregnancy, normal
20	R. H.	0.346%	0.342%	0.344%	Pregnancy, normal
21	A. DeC.	0.383%	0.398%	0.390%	Pregnancy, normal
22	E. S.	0.255%	0.243%	0.249%	Pregnancy, normal
23	A. D.	0.393%	0.400%	0.397%	Pregnancy, normal, second month
24	A. D.	0.181%	0.188%	0.185%	Pregnancy, normal, fourth month
25	A. D.	0.411%	0.406%	0.409%	Pregnancy, normal, fifth month
26	A. D.*	0.417%	0.401%	0.409%	Pregnancy, normal, seventh month
27	R. G.	0.434%	0.474%	0.454%	Pregnancy, hypertension
28	L. E.	0.447%	0.494%	0.471%	Pregnancy, hypertension
29	F. D.	0.472%	0.468%	0.470%	Pregnancy, toxemia
30	C. R.†	0.413%	0.421%	0.416%	Pregnancy, thyrotoxicosis
31	L. A.	0.875%	0.988%	0.932%	Pregnancy, post cesarean phlebitis
32	H. W.	0.494%	0.461%	0.478%	Pregnancy, placenta previa
33	E. G.	0.502%	0.486%	0.494%	Pregnancy, placenta previa
34	F. G.	0.420%	0.414%	0.417%	Pregnancy, fourth month, psychosis
35	N. T.	0.632%	0.619%	0.625%	Pregnancy, eclampsia
36	C. P.	0.644%	0.644%	0.644%	Pregnancy, eclampsia
37	M. P.	0.632%	0.673%	0.653%	Pregnancy, gastrointestinal
38	Mr. S.	0.137%	0.140%	0.139%	Banti's disease
39	Mr. S.	0.131%	0.137%	0.134%	Banti's disease

*Third Value 0.409%
†Third Value 0.415%

Experiment showed that, with normal bloods at least, clotting was complete in thirty minutes as evidenced in Table II. This is also stated to be the case by Foster and Whipple.[6]

TABLE II

EFFECT OF VARYING CLOTTING TIME. VALUES IN PER CENT PLASMA FIBRIN. TIME IN MINUTES

NAME		30	60	120	180	240	MINUTES
F.	1.	0.314	0.300	0.308	0.274	0.300	
	2.	0.318	0.290	0.305	0.300		
	Mean	0.316	0.295	0.307	0.287	0.300	
C.	1.	0.266	0.267	0.264	0.266	0.266	
	2.	0.265	0.265	0.265	0.257	0.263	
	Mean	0.266	0.266	0.265	0.262	0.264	
W.	1.	0.283	0.262	0.268	0.276	0.267	
	2.	0.273	0.261	0.265	0.286		
	Mean	0.278	0.262	0.267	0.281	0.267	

In the case of only one plasma sample has clotting failed to take place, and in this case the mixture remained entirely liquid after several hours' standing and the addition of calcium chloride a second time.

If it were the universal custom to use the Kjeldahl method for the determination of fibrin, it would seem logical to express results as fibrin nitrogen rather than as fibrin. But in view of the large number of published results obtained by gravimetric methods and the continued use of this procedure, it has seemed best to tabulate results as fibrin. The fibrin values have been obtained by multiplying the nitrogen values by 6.25. While Hammarsten gives the percentage of nitrogen in horse fibrin as 16.91 per cent, which would give a factor of 5.91, in the absence of any recent study of the nitrogen percentage of human fibrin, it has seemed best to employ the conventional factor 6.25.

It is the custom of many workers to calculate the fibrin concentration on the basis of whole blood, the corpuscle-plasma ratio being determined by the hematocrit method. In my opinion, such calculations are of questionable accuracy. While in one paper[6] it is stated that it is possible to express all plasma from between the corpuscles by centrifuging at 3000 R. P. M. for thirty minutes, it would seem that this statement is open to question. In my opinion it is much better to express the fibrin concentration in terms of plasma. All results in this paper, therefore, are expressed as per cent plasma fibrin, that is, grams of fibrin (fibrin nitrogen × 6.25) per 100 c.c. of plasma.

DETAILS OF PROCEDURE

Blood is drawn from the vein by means of a syringe and introduced at once into a bottle previously prepared with the required quantity of solid lithium or potassium oxalate by measuring into it that volume of a standard solution of the oxalate required to furnish 1 mg. of the lithium salt or 2 mg. of the potassium salt per c.c. of blood. Water is expelled by drying in an oven. After addition of the blood, a rubber stopper is inserted into the bottle and the contents shaken thoroughly but not violently, as violent shaking

sometimes causes laking; this tends to contaminate the fibrin with corpuscle protein and give results above the true value. The blood is now centrifuged for twenty to thirty minutes at about 2000 R. P. M. and the plasma carefully drawn off with a medicine dropper with a fine tip into another centrifuge tube. It is much more satisfactory to remove the plasma in this manner than to attempt to measure with a pipette directly from the centrifuge tube containing both plasma and corpuscles.

One c.c. of clear plasma is measured with a calibrated Ostwald-Folin pipette into a 25 × 200 mm. test tube, 28 c.c. of 0.8 per cent NaCl and 1 c.c. of 2.5 per cent $CaCl_2$ are added and the contents mixed and allowed to stand for at least thirty minutes. At the end of that time a rather firm jelly should be formed. The jelly is loosened by inclining and rotating the tube and the contents poured upon a filter which has previously been moistened with 0.8 per cent NaCl and attached to the funnel. A glass rod drawn out to a diameter of approximately 1.5 mm. and pointed, is introduced, and by rotating the rod slowly the fibrin is collected quantitatively on the end of the rod as a firmly adhering mass. The fibrin is washed with distilled water from a wash bottle, dried with filter paper, carefully removed from the rod by means of filter paper and introduced into a 25 × 200 mm. Pyrex tube graduated at 50 c.c. One c.c. of the concentrated digestion mixture of Folin* (100 c.c. concentrated H_2SO_4, 300 c.c. 85 per cent H_3PO_4, 50 c.c. 5 per cent $CuSO_4.5H_2O$) is added, the mixture heated gently with a micro Bunsen burner until the fibrin is dissolved, then more strongly until the tube is filled with white fumes when a small watch glass is placed over the mouth of the tube and the heating continued for thirty seconds after the contents of the tube have become a light bluish shade with no trace of brown. After cooling for two minutes, distilled water is added to approximately 40 c.c., the contents of the tube stirred with a long glass rod to break up any mass of silica formed, and the rod rinsed with distilled water. When the contents of the tube have cooled to room temperature, the volume is made up to 50 c.c. with distilled water and thoroughly mixed. The contents of the tube are now poured into a 50 c.c. conical centrifuge tube and centrifuged until the silica is all precipitated and the supernatant liquid entirely clear, which usually requires from twenty to thirty minutes. Twenty-five c.c. of the clear supernatant liquid are pipetted into a 50 c.c. volumetric flask, 15 c.c. of the Nessler's solution described by Folin and Wu[12] are added, and the contents made up to volume, mixed and compared in a colorimeter with a standard ammonium sulphate solution, the standard being set at 20.0 mm. A 0.5 mg. nitrogen standard is used which is prepared by measuring 5 c.c. of the standard ammonium sulphate solution described by Folin and Wu[12] (0.4716 gm. dry C.P. $(NH_4)_2SO_4$ per liter) into a 100 c.c. measuring flask, diluting with 60 c.c. distilled water, adding 1 c.c. of the concentrated digestion mixture and nesslerizing with 30 c.c. Nessler's solution. If perferred, the silica may be removed by filtering through a Munektell OB or other similar retentive filter into a 100 c.c. volumetric

*The digestion cannot be carried out successfully with the dilute digestion mixture used in the N. P. N. method of Folin and Wu as the fibrin does not dissolve readily in this mixture, and during the heating becomes disintegrated and is apt to be spattered on the sides of the tube to which it adheres firmly.

flask, washing to a volume of about 60 c.c., and nesslerizing with 30 c.c. Nessler's solution. In either case the calculation is made as follows:

$$\text{Per cent plasma fibrin} = \text{gm. fibrin per 100 c.c. Plasma} = \frac{\text{Standard Reading}}{\text{Sample Reading}} \times 0.5 \times 6.25$$

While the agreement between duplicate determinations is good, it is always advisable to carry out two determinations on each sample of plasma and use the mean value. This can be done with 5 c.c. of whole blood.

SUMMARY

Details have been given of a method for the determination of fibrin in blood plasma which requires only a small volume of blood and which is believed to offer a satisfactory degree of both ease and accuracy.

REFERENCES

[1]Meek, W. J.: Am. Jour. Physiol., 1912, xxx, 161.
[2]Starlinger, W.: Biochem. Ztschr., 1923, cxl, 203.
[3]Cullen, G. E., and Van Slyke, D. D.: Jour. Biol. Chem., 1920, xli, 587.
[4]Howe, P. E.: Jour. Biol. Chem., 1921, xlix, 109.
[5]Gram, H. C.: Jour. Biol. Chem., 1921, xlix, 279.
[6]Foster, D. P., and Whipple, G. H.: Am. Jour. Physiol., 1923, lviii, 365.
[7]Winternitz, P.: Arch. Derm. Syph., 1910, ci, 227.
[8]Leendertz, G., and Gromelski, B.: Arch. exper. Path. u. Pharmakol., 1922, cxiv, 114.
[9]Leendertz, G.: Klin. Wchnschr., 1923, ii, 746.
[10]Wohlgemuth, J.: Biochem. Ztschr., 1910, xxv, 79.
[11]Wu, H.: Jour. Biol. Chem., 1922, li, 33.
[12]Folin, O., and Wu, H.: Jour. Biol. Chem., 1919, xxxviii, 81.

A DEVICE FOR THE DILUTION OF ANTIGEN IN THE KAHN PRECIPITATION TEST*

By Herbert Silvette, Richmond, Virginia

IT IS somewhat difficult without experience to dilute the antigen with saline in the Kahn Test without loss of fluid by splashing and without the separation of cholesterin crystals on account of too slow mixing. The device herein described may be used with the confidence that the dilution will be correctly made, without either of the above faults.

The device is made from an ordinary test tube, size 15 x 150 mm. The first step in the procedure is to blow a hole in the wall of the tube at a point about 2 or 3 cm. from the closed end. This is done by heating the wall red hot at one point with a hot flame and blowing sharply into the other end with a rubber bulb (Fig. 1.). A little further application of the flame finishes off the hole nicely. Then the other end of the tube is drawn out slightly and bent sharply upwards, so that the mouth of the tube now points in the same direction as the

*From the Pathological Laboratory, Johnston-Willis Hospital.
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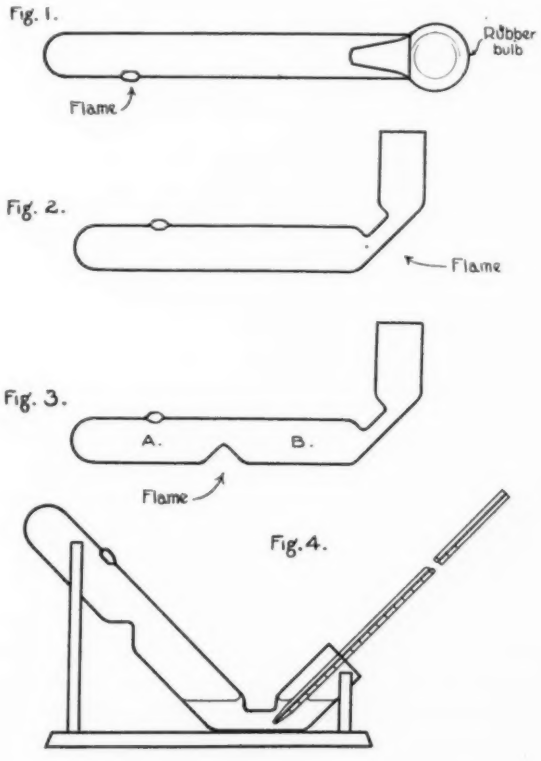
opening (Fig. 2). Finally, the wall of the tube is heated red hot at a point mid-way between the closed end and the neck and on the opposite side of the tube from the hole. The tube is quickly pressed down upon the sharp edge of a warmed three-cornered file so that a partition is formed within the tube (Fig. 3). By varying the height of this partition, the tube may be made to dilute 1, 2, or even 3 c.c. of antigen.

In using, an amount of antigen is placed into compartment *A* through the hole, and an amount of saline (as determined by titration) is gently run down



the neck into *B*. The tube is then sharply tilted so that the saline runs into the antigen compartment, and immediately the tube is rocked back and forth a few times, thus insuring complete mixing of the contents. When the hole is correctly placed, there will be no danger of the antigen dilution splashing through. The apparatus must, of course, be both clean and dry before using. This may be accomplished by rinsing the tube first with water, then with alcohol, and allowing it to drain over night.

The antigen dilution after standing may be pipetted directly from the tube (Fig. 4), or the contents may be emptied into a standard antigen dilution tube.

NOTE ON URINE PRESERVATIVES*

By J. J. Short, M.D. and A. Piatetzky, M.D., New York, N. Y.

THE requirements for an ideal urine preservative are summarized by Behre and Muhlberg[1] as follows:

"1. It should preserve the urine from bacterial decomposition and the development of moulds or other growths for considerable periods of time under average conditions.

"2. It should not interfere either positively or negatively with any of the physical, chemical or microscopic tests in ordinary use.

"3. It should be readily soluble.

"4. It should not interfere to any marked extent with the normal reaction of the urine.

"5. It should be a solid.

"6. Its cost should be reasonable."

We have conducted the search for such a preservative at varying intervals since early in 1925. Of the preservatives reported by Behre and Muhlberg we have tried boric acid, borax, toluene, thymol, resorcinol, salicylic acid, sodium benzoate, and urofix, with results very similar to theirs. It will not, therefore, be necessary to report in detail on these substances. Details of other substances and combinations we have tried follow.

EXPERIMENTAL

Our tests were made on 30 c.c. urine specimens at incubator temperature; unpreserved specimens and specimens containing well-known preservatives were used as controls. These were examined daily for several days, and the odor, color and sediment noted. Occasionally cultures were made on blood agar plates. The specimens were also centrifuged and sediments examined microscopically where results were promising as judged by gross inspection.

Nitro-benzene, a liquid, was one of the first substances tried. This gave poor preservation alone, excellent preservation when combined with boric acid.† Only about 25 milligrams of each were necessary. It was discarded because the two substances could not be combined in tablet form.

Di-nitro-benzene, a solid, showed good preservation when combined with boric acid.† As it is only slightly soluble and leaves a sediment of its own it was discarded.

Nitro-phenol, and several other aromatic nitro-compounds were tried and found unsatisfactory for various reasons.

*Experimental work described in this paper was done at the Laboratories of the Life Extension Institute, New York.
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†An additional reason for discarding boric acid is that it interferes with the Benedict picric acid sugar determination as reported by Behre and Muhlberg.

Benzoic acid alone was found to be inadequate in preserving action, although superior to sodium benzoate.

Sodium bisulphite aroused our interest for some time, as it met all requirements in the incubator tests. The urine was somewhat bleached but was otherwise uninterfered with, and was promptly sterilized. In routine use, however, urines were not preserved due to the oxidation of bisulphite to sulphate when exposed to the air. It therefore had to be discarded.

*Chinosol** was found to be one of the most satisfactory preservatives that we have tried. About 20 mg. only are necessary for a 30 c.c. specimen. It colors the urine slightly but does not interfere with any of the routine tests.

Several mercury compounds and various other substances tried by us did not give sufficiently promising results to be worth mentioning.

Hexamethylenamine.—Since the paper of Behre and Muhlberg we have turned our attention to hexamethylenamine and share their opinion that it is, of all tried, the most satisfactory. They advised combining powdered hexamethylenamine with salicylic acid in the proportion of 3 to 2; 50 mg. of such intimate mixture were then added to each 10 c.c. of urine. This would be the equivalent of 90 mg. of hexamethylenamine to 60 mg. of salicylic acid for the preservation of a 30 c.c. specimen. The chief objection to the preservative advocated by Behre and Muhlberg was their inability to put their mixture in tablet form. We made such an attempt and likewise failed due to the extreme bulkiness and lightness of salicylic acid. As a substitute for salicylic acid therefore we turned to acetyl-salicylic acid which has quite different physical properties. Theoretically it seemed to us that this should be equal to salicylic acid for our purpose as it is more soluble in water, and in contact with moisture decomposes into salicylic acid and acetic acid. Actual test proved this to be so, as it gave excellent preservation when added to urine with hexamethylenamine in practically the same proportions suggested by Behre and Muhlberg for hexamethylenamine and salicylic acid. We have tested the effect of this combination on nearly all the various routine tests employed today in urinalyses and find that it interferes with only one—the ferric chloride test for acetoacetic acid. This test, however, is made only occasionally in routine work (when diabetes is suspected), and there can be no falsely positive report for this substance if one tries the effect of heat on the dark color produced, and checks the result with the sodium nitroprusside test for acetone. Although it is well known that the ingestion of coal tar products frequently causes the appearance of copper reducing substances in the urine, it has been shown by Leas[2] that such reduction is not due to the presence of the coal tar derivatives themselves but to some excreted substance resulting from their ingestion. Sodium salicylate gave no reduction when added directly to urine in their experiment. The same was true of acetyl-salicylic acid in our tests. Attempts to combine hexamethylenamine and acetyl-salicylic acid in a single compressed tablet likewise resulted unsatisfactorily. Although this is possible and a good hard tablet is produced, we found that the proportions of the two ingredients varied due to their difference in physical properties. Furthermore on standing the tablets became hygroscopic and dissolved after a short period of time. Finally we decided on

*A product manufactured by Parmele Pharmacal Co., 47 West Street, New York, N. Y.

the practical necessity of two tablets, one to contain hexamethylenamine and one acetyl-salicylic acid. For the preservation of 30 c.c. specimens we therefore make a 100 mg. compressed tablet of hexamethylenamine and another such tablet containing 50 mg. each of acetyl-salicylic acid and potassium nitrate, the latter an inert substance used merely for the purpose of facilitating the feeding of the acetyl-salicylic acid into the compressing machine.

COMMENT

The use of any preservative not in tablet form is time-consuming. Although it would have been more desirable to have the preservative in the form of a single tablet we find that with a little practice two small tablets can be added almost as quickly as a single tablet, one tablet being added simultaneously from each hand. The substances described above are ideal for use in a tablet compressing machine as they feed regularly and give tablets of exceedingly uniform weight. Both tablets go into solution readily. The cost is extremely low when compared with that of the various urine preservatives now being marketed.

REFERENCES

[1]Behre, Jeanette Allen, Ph.D. and Muhlberg, William, M.D.: Urinary Preservatives Including Hexamethylenamine, Jour. Lab. & Clin. Med., May, 1926, xi, No. 8, p. 785.
[2]Leas, R. D.: Reducing Substances in the Urine, Jour. Lab. & Clin. Med., October, 1920, xii, No. 1, p. 15.

THE BLOOD-URINE DENSITY INDEX*

By David Polowe, M.D., Paterson, N. J.

A NEW index, the Blood-Urine Density Index (B-U DI) is herewith described. It is intended to serve as a rapid orientation index of the blood-urine density balance. Less than ten minutes are required to make the determination in any given case, the falling drop method of Barbour and Hamilton[1] being used.

Only one author, Schmaltz[2] in 1891, so far as I have been able to discover, while determining the influence on blood density following the imbibition of great quantities of 0.6 per cent saline, made annotations as to the specific gravity of the excreted urine. He may have had some blood-urine density balance in mind but he made no comments on this phase of the matter.

BLOOD DENSIMETRY ON EXPERIMENTAL ANIMALS

Copeman,[4] in 1891, ligated an extremity on each of four rabbits. The density of the blood distal to the ligature was determined. Both the red cell count and the blood density were increased in three rabbits. In the fourth rabbit, the arterial supply being also shut off, no change was found.

Popel,[6] in 1895, working on dogs, found that the imbibition of water low-

*From the Barnert Memorial Hospital.
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ers blood density, abstinence increases it, while ureteral ligation plus abstinence markedly increases it.

Barbour and Hamilton,[5] studying emotional anhydremia, have shown that "* * * excitement always increases the blood concentration, sometimes by as much as 10 per cent." This is well to remember when dealing with needle-shy patients.

TABLE I

COPEMAN'S COMPOSITE TABLE OF BLOOD DENSITY IN DISEASE

DISEASE	COPEMAN	QUINKE	BECQUERAL AND RADIER
Anemia chlorosis	1041– 1043	10352–1049	10458 (mean of 6)
Pernicious	1027– 1034		
Leucocythemia	10485–10510	10443	1036–10495 (5 cases)
Gastric ulcer	1038		
Lymphadenoma	1062		
Hemoglobinuria	10503–10520		
Scurvy		10608	
Cardiac	10495–10520	1058 (angina)	1050–10525 (55 cases)
Diabetes mellitus	10585 (2 cases)	10549–10595	
Cirrhosis of liver	10520	10496 (hemophilia)	
Acute nephritis	10570		
Chronic nephritis	10545–10600	10473–10487	
Uremia	1052	10505	
Tuberculous kidney	10485		
Tuberculous peritonitis	10570		
Typhoid fever		10544–10621	
Cerebrospinal meningitis		10579	
Pyemia		10505	
Chorea	10530		
Rheumatism and hysteria	10585		

TABLE II*

BLOOD DENSITY IN DISEASE

AGE	SEX	DIAGNOSIS	BLOOD DENSITY
22	F	Tuberculosis, pulmonary	10360
20	F	Tuberculosis, pulmonary	10550
48	F	Tuberculosis, pulmonary, plus myoma uteri	10560
35	F	Chyluria	10450
15	F	Chlorosis	10440
62	F	Carcinoma of stomach	10390
34	F	Anemia	10580 (?)
37	M	Mitral stenosis	10570
51	F	Aortic insufficiency	10560
57	F	Syphilis	10510
30	F	Syphilis	10560
79	M	Syphilis, old. Senile marasmus	10520
73	F	Senile marasmus	10507
79	M	Senile marasmus	10520

*From Schmaltz.

THE CLINICAL SIGNIFICANCE OF BLOOD DENSITY

This phase of clinical medicine has been neglected, due possibly to the technical difficulties with the older methods. Copeman, however, did a considerable amount of work along these lines and his composite table of blood density in disease appears in Table I.

Schmaltz studied the influence of large quantities of water, the influence of food, exercise, warm baths, menses, age, and the time of the day when the density is determined. Table II indicates his findings in diseases. He con-

cludes his paper by stating that blood density varies within narrow limits in health and varies considerably under pathologic conditions.

Lyonnet,[3] in 1892, cites Lloyd Jones' findings in nephritis, the blood density in parenchymatous nephritis being 1034, in interstitial nephritis 1062 to 1042. A differential diagnosis between cardiacs and nephritics is suggested in that the blood density on cardiacs is about 10594, in nephritics 10516.

Bender and Polowe,[12] in a series of eight spinal anesthesias, have found that with the fall in blood tension that follows the administration of the spinal

TABLE III*

BLOOD DENSITY AND BLOOD TENSION DURING SPINAL ANESTHESIA†

TIME A.M.		TENSION	FALLING TIME		BLOOD DENSITY
BP	BD		BLOOD	STANDARD	
9:57		104/72	17.7	18.6	10560
10:03	10:05	88/64	20.4		10533
	10:09		21.9		10529
10:14	10:16	84/62	21.7	18.5	10531
	10:25		23.0		10511
10:28	10:29	84/60	20.8		10527
10:35	10:36	84/64	20.9	18.4	10526
10:45	10:46	86/64	20.6		10529
	10:57		20.5		10530
10:58	10:59	154/92	20.4		10531
11:06	11:07	182/98	19.9		10535
11:08	Operation ended				

*Bender and Polowe[12].

†Case 1, October 1, 1926. Female, aged fifty. Vaginal repair. Control (prior to operation) B. P. 172/78, B. D. 10552.

anesthetic there is a concomitant fall in blood density. Table III illustrates one such case, it being noted that as the patient begins to react from the anesthesia the rise in blood tension is followed by an increase in blood density. Further data is to be collected on this phase of blood densimetry and will be published in another paper.

METHODS FOR DETERMINING BLOOD DENSITY

Three principle methods for determining blood density have been employed. These are (a) the pycnometer method, flask or capillary, the latter being used by Schmaltz; (b) permitting a drop of blood to fall into a fluid of known density, a technic used by Hammershlag, Copeman, and more recently by Kirkpatrick and Kling;[8] (c) the falling drop method of Barbour and Hamilton who state: "*Principle:* A drop of blood (or other body fluid) of definite size is released below the surface of a nonmiscible mixture. Its rate of fall depends on its density, which can be easily calculated as soon as the rate of fall of a similar drop of standard solution of known density (released under identical conditions) is available for comparison." For details of apparatus, material, and procedure the reader is referred to the recent publications of Barbour and Hamilton.[1, 5]

THE BLOOD-URINE DENSITY INDEX

The density of the blood is determined by the falling drop method of Barbour and Hamilton. The density of the urine is determined in the same

way, or by urinometer, immediately before or after that of the blood is determined.

The calculation of the blood-urine density index (B-U DI) is made by taking the values to the right of the decimal point, the blood value being divided by the urine value. *Example:* BD = 1060; UD = 1020; then 60/20 = 3 which is the blood-urine density index (B-U DI).

NORMAL VALUES

Normal Blood Densities.—Fifty-two observations, by the method proposed in this paper, on ten normal cases are reported in Table V. These values are in fair agreement with those of Bamberger (cited by Lyonnet) and Schmaltz. Normal blood densities range between 1050 and 1060, are higher in males, and higher in the morning than in the afternoon.

Normal Urine Densities.—It is important to note the values recorded in Table IV for the age groups between one day and fourteen years.

TABLE IV

NORMAL BLOOD-URINE DENSITY INDICES

AGE GROUPS	BLOOD DENSITY	URINE DENSITY	B-U DI
1 to 3 days	1060–1080*	1010–1012†	5– 8
4 to 10 days	1060–1080*	1004–1008†	8–20
10 days to 6 mo.	1053–1059*	1004–1010†	5–15
6 mo. to 2 yr.	1053–1059*	1006–1012†	4–10
2 yr. to 8 yr.	1056–1060*	1008–1016†	4– 8
8 yr. to 14 yr.	1056–1066*	1012–1020†	3– 5
14 yr. up	1050–1060	1015–1025	2– 4

*Burton—From Burton-Opitz[10].
†From Holt and Howland[9].

BLOOD-URINE DENSITY INDICES IN DISEASE

Thirty-nine cases, on whom 63 observations were made, are reported. Fifteen cases of diabetes mellitus are recorded in Table VI. Observations in one case of carcinoma of the bladder are recorded in Table VII. Five kidney cases are grouped in Table VIII. The other 18 cases are grouped in Table IX.

Cases 8 and 9 (Table VI) were hospital cases. The rest in that table were clinic patients attending the diabetic clinic in the Barnert Memorial Hospital. Cases 8 and 9 were carefully dieted and insulin administered when indicated. Both cases improved and with the improvement there was an increase in the B-U DI, which is suggestive of the prognostic value of the index. That normal urine densities may be associated with glycosuria is in agreement with the findings of Joslin.[11] It is also of interest to note that while the B-U D indices below 2 occur in about 33 per cent of the observations, these are not always associated with a high urine density as might be expected from a priori reasoning. Attention is also called to the hydremic condition of the blood as evidenced by the low blood densities in 37 per cent of the observations.

Very low indices were found in both cases of carcinoma. Case 28 was diagnosed as benign prostatic hypertrophy. Concentration and dilution tests were run simultaneously with B-U DI determinations. The urine density fluctuations were compatible with operation. The B-U DI findings were decidedly against operation. Cystoscopy was unsatisfactory. At operation an

TABLE V

NORMAL BLOOD DENSITIES AS FOUND BY POLOWE

CASE	ROOM TEMP. C.	DATE	AGE	SEX	TIME*	BLOOD STAND. FALLING TIME		BLOOD DENSITY
1	23.0	8/27	33	M	A.M.	18.5	20.3	10573
	23.0	8/28	33	M	A.M.	19.5	20.3	10565
	26.5	8/29	33	M	A.M.	17.5	18.3	10560
	24.5	8/30	33	M	A.M.	18.8	20.1	10562
	22.0	9/2	33	M	P.M.	26.6	22.8	10534
2	23.0	8/28	23	M	A.M.	16.2	20.3	10600
	26.5	8/29	23	M	A.M.	14.6	18.3	10606
	24.5	8/30	23	M	A.M.	17.0	20.1	10586
	24.5	8/31	23	M	P.M.	20.9	19.5	10537
3	25.5	8/26	29	F	A.M.	21.5	18.5	10523
	24.0	8/27	29	F	A.M.	18.9	20.7	10565
	24.0	8/28	29	F	A.M.	20.5	20.0	10545
	22.0	9/2	29	F	P.M.	29.3	22.8	10528
4	25.5	8/26	26	F	A.M.	20.9	17.8	10521
	23.0	8/27	26	F	A.M.	23.9	20.5	10526
	24.0	8/28	26	F	A.M.	22.6	20.0	10530
	26.5	8/29	26	F	A.M.	20.7	18.3	10527
	24.5	8/30	26	F	A.M.	24.6	20.1	10518
	22.0	9/2	26	F	P.M.	30.4	22.8	10513
5	25.5	8/26	19	F	A.M.	18.1	17.8	10546
	24.5	8/27	19	F	A.M.	17.3	19.9	10580
	24.5	8/30	19	F	A.M.	21.4	20.1	10538
	24.5	8/31	19	F	P.M.	25.0	19.5	10510
6	25.5	8/26	18	F	A.M.	17.9	18.2	10553
	24.0	8/27	18	F	A.M.	21.6	20.5	10539
	23.0	8/28	18	F	A.M.	22.0	20.3	10536
	26.5	8/29	18	F	A.M.	20.4	18.3	10529
	24.5	8/30	18	F	A.M.	22.0	20.1	10534
	24.5	8/31	18	F	P.M.	25.1	19.5	10511
	22.0	9/2	18	F	P.M.	24.3	22.8	10540
7	25.5	8/26	28	F	A.M.	18.5	18.2	10545
	22.5	8/27	28	F	A.M.	21.5	20.5	10541
	24.5	8/28	28	F	A.M.	20.4	19.9	10544
	26.5	8/29	28	F	A.M.	18.0	18.3	10554
	24.5	8/30	28	F	A.M.	18.7	20.1	10564
	24.5	8/31	28	F	P.M.	19.6	19.5	10549
8	25.5	8/26	32	F	A.M.	20.1	18.2	10530
	24.0	8/27	32	F	A.M.	19.3	20.7	10564
	24.5	8/28	32	F	A.M.	18.5	19.9	10562
9	25.5	8/26	33	F	A.M.	19.1	18.2	10541
	24.0	8/27	33	F	A.M.	18.5	20.1	10565
	23.0	8/28	33	F	A.M.	22.7	20.3	10538
	26.5	8/29	33	F	A.M.	20.1	18.3	10530
	24.5	8/30	33	F	A.M.	22.9	20.1	10527
	24.5	8/31	33	F	P.M.	25.2	19.5	10510
10	25.4	8/26	18	F	A.M.	18.4	18.2	10547
	22.5	8/27	18	F	A.M.	19.9	20.7	10557
	24.5	8/28	18	F	A.M.	19.0	19.9	10560
	26.5	8/29	18	F	A.M.	22.1	18.3	10514
	24.5	8/30	18	F	A.M.	18.5	20.1	10567
	24.5	8/31	18	F	P.M.	20.0	19.5	10545
	22.0	9/2	18	F	P.M.	24.5	22.8	10539

*A.M. = Between 9 and 10 o'clock.
P.M. = Between 2 and 4 o'clock.

TABLE VI

BLOOD-URINE DENSITY INDICES IN DIABETES MELLITUS

CASE	DATE	AGE	SEX	BLOOD DENSITY	URINE DENSITY	B-U DI	GLYCOSURIA	REMARKS
8	11/27	60	F	10536	1020	2:68	No	Insulin given
	12/6			10527	1017	3:10	No	Insulin given
9	11/27	60	F	10533	1029	1:84	No	No insulin given
	12/6			10506	1026	1:95	No	No insulin given
	12/13			10502	1022	2:28	No	Insulin given
10	11/30	40	M	10570	1037	1:54	Yes	Patient emaciated. Blood sugar 273
11	11/30	40	F	10538	1015	3:59	No	Blood sugar 215
12	11/30	50	F	10543	1017	3:19	Yes	Insulin given
	12/14			10497	1020	2:49	Yes	Insulin given
13	11/30	55	F	10557	1025	2:23	Yes	Insulin given
	12/7			10543	1012	4:52	No	Insulin given
14	12/7	40	F	10510	1020	2:59	Yes	Insulin given
	12/14			10475	1031	1:53	Yes	Insulin given
15	12/7	40	F	10553	1022	2:51	Yes	No insulin given
	12/14			10412	1016	2:58	Yes	No insulin given
16	12/7	50	F	10487	1020	2:44	Yes	No insulin given
17	12/14	40	F	10318	1020	1:59	Yes	No insulin given
18	12/14	60	M	10526	1022	2:39	No	No insulin given
19	12/14	60	F	10533	1020	2:67	Yes	No insulin given
20	12/14	25	F	10392	1027	1:45	Yes	Patient obese. Insulin given
21	12/14	60	M	10398	1023	1:72	No	No insulin given
22	12/14	50	F	10474	1016	2:96	Yes	No insulin given

TABLE VII

CARCINOMA OF THE BLADDER*

TIME	ROOM TEMP. C.	URINE EXCRETED	URINE DENSITY	BLOOD DENSITY	B-U DI
8:30 A.M.	24.5	100 c.c.	1020		
9:30 A.M.		50 c.c.	1024		
10:00 A.M.		75 c.c.	1026	10360	1:39
10:30 A.M.		50 c.c.	1025	10385	1:54
11:10 A.M.		50 c.c.	1027	10353	1:31
12:00 A.M.		90 c.c.	1015		
12:20 P.M.				10382	2:55
2:10 P.M.		50 c.c.	1020	10398	1:99
4:00 P.M.		200 c.c.	1014	10403	2:88
5:20 P.M.				10433	
6:00 P.M.		100 c.c.	1015		2:89
7:45 P.M.		50 c.c.	1022	10357	1:67
8:00 A.M.	12/14	400 c.c.	1024	10350	1:46

*This renal function test, used in Vienna on prostate cases, was introduced in the Barnert Memorial Hospital by Dr. D. H. Mendelsohn of the Surgical Division.

TABLE VIII

BLOOD-URINE DENSITY INDICES IN 5 KIDNEY CASES*

CASE	DATE	AGE	SEX	BL DN	UR DN	B-U DI	NPN	UN	KRYOSCOPY	DIAGNOSIS; REMARKS
23	11/24	35	M	10465	1020	2:32				Polycystic kidneys
24	12/6	40	M	10533	1012	4:44	59	27		Chr. nephritis
25	11/27	35	M	10374	1012	3:12	48	24		Ac. rh. fev. w ac. neph.
	12/6			10393	1024	1:63		12/10	0.59	
	12/13			10364	1026	1:40				
	12/22			10365	1011	3:32				Clinically improved
26	12/7	50	F	10288	1016	1:80				Atrophy. rt. k. congenital
	12/13			10302	1020	1:51	26	13		Nephrectomy done
43	3/8/27	60	M	10286	1006	4:77	109	75		Chr. neph.; died 3/13/27

*NPN, UN, kryoscopy, and blood sugar values in this paper were obtained from Dr. H. Wassing, pathologist, Barnert Memorial Hospital.

TABLE IX

BLOOD-URINE DENSITY INDICES IN 18 HOSPITAL CASES

CASE	DATE	AGE	SEX	BLOOD DENSITY	URINE DENSITY	B-U DI	DIAGNOSIS; REMARKS
5	12/22	30	F	10326	1015	2:17	Hysteria; sec. anemia
7	12/22	30	F	10372	1020	1:86	Internal hemmorrhoids
27	11/27	45	M	10373	1034	1:09	Ca. pancreas; late
29	11/27	25	M	10430	1026	1:65	Tb. pulm.; effusion
	12/6			10372	1023	1:63	
	12/13			10339	1026	1:21	
30	12/22	45	M	10501	1031	1:62	Tb. pulmonary
31	11/27	45	F	10509	1007	7:27	Aur. fibril.; decompensated
32	12/22	15	F	10497	1028	1:77	Tricus. and mitral.; decomensated
33	11/27	15	F	10470	1010	4:70	Rheum. pneumonia; mitral
	12/6			10505	1018	2:81	Improved
34	11/27	65	F	10541	1015	3:61	Hemiplegia, chronic
	12/6			10511	1014	3:64	
35	12/6	60	F	10557	1026	2:14	Cerebral apopl. coma, died
36	12/6	15	M	10537	1024	2:24	Fr. skull, recovered
37	12/6	60	M	10551	1018	3:10	Prostatic hypertr., benign.
38	12/6	50	M	10513	1020	2:56	Pneumoperitoneum, irratnl.
39	12/13	90	M	10473	1016	2:96	Senility, NPN 39; UN 22
40	12/22	30	F	10319	1019	1:68	Stric. rectum, syphilis, died
41	12/24	75	M	10340	1016	2:12	Portal cirrhosis; ascites
42	11/27	40	F	10488	1014	3:49	No diag. observation
44	3/10/27	45	F	10550	1015	3:67	Chronic myocarditis Kyphosis, died 3/11/27

extensive carcinoma of the bladder was found. This phase of B-U DI determinations is being studied further in the hope that it may be helpful in solving the problem of operability in prostate, bladder, and kidney conditions.

Case 32 (Table IX) was admitted as a cardiac with mitral and tricuspid lesions, edema, and a pulsating liver. Her B-U DI was below 2. This may indicate that B-U DI findings in children are of greater significance when below the normal than when above it for the prescribed age.

COMMENT

The facts presented here are far too insufficient for one to draw any conclusions as to the true clinical value of the Blood-Urine Density Index. At present, when this index is used in conjunction with other available clinical and laboratory facts, it appears to help round out the clinical picture. Values above 4, in adults, seem to suggest intranephritic pathology. Values below 2 point toward a blood urine imbalance which seems to be primarily of extranephritic origin. When the B-U DI hovers about 1.5 or less malignancy should be considered as a possibility.

SUMMARY

1. A new index, known as the Blood-Urine Density Index (B-U DI), is described.

2. The density of the respective fluids is obtained by the falling drop method of Barbour and Hamilton for blood, and by the same method (or by urinometer as in the cases herein reported) for urine.

3. Normal indices for adults lie between 2 and 4. For infants and children they range from 3 to 20.

4. Pathologic indices for infants and children seem to lie below the normal for the given age group. Pathologic indices for adults lie below 2 and above 4.

REFERENCES

[1]Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity of Fluids; Journal of Biological Chemistry, August, 1926, lxix, 625.

[2]Schmaltz, von Dr. Richard: Die Untersuchung des Specifischen Gewichtes des Münchlichen Blutes; Deutsch. Arch. f. klin. Med., 1891, xlvii, 145-158.

[3]Lyonnet, Le Dr. B.: De La Densité Du Sang: Sa Détermination Clinique; Ses Variations Physiologiques et Pathologiques, Paris; J. B. Balliere et Fils, 1892.

[4]Copeman, S. Monkton, M.A., M.D., Contab: Report on the Specific Gravity of the Blood in Disease; Brit. Med. Jour., Jan. 24, 1891, i, 161.

[5]Barbour, H. G., and Hamilton, W. F.: Jour. Am. Med. Assn., Jan. 8, 1927, lxxxviii, 91.

[6]Popel, W.: Sur les Variations de la Densité Du Sang Dans le Jeune Absolu, Simple, ou Complique de la Ligature Des Ureteres; Arch. d. Sc. Biol., 1895-1896, iv, 345-760.

[7]Barbour, H. G., and Hamilton, W. F.: Am. Jour. Physiol., 1924, lxix, 654.

[8]Kirkpatrick, John, and Kling, David H.: A Simplified Method for Determination of the Specific Gravity of a Few Drops of Urine; Jour. Am. Med. Assn., August 14, 1926, lxxxvii, 487.

[9]Holt, L. Emmett, and Howland, John: The Diseases of Infancy and Childhood, ed. 8, p. 592.

[10]Burton-Optiz, Russell: Textbook of Physiology, 1920, p. 163.

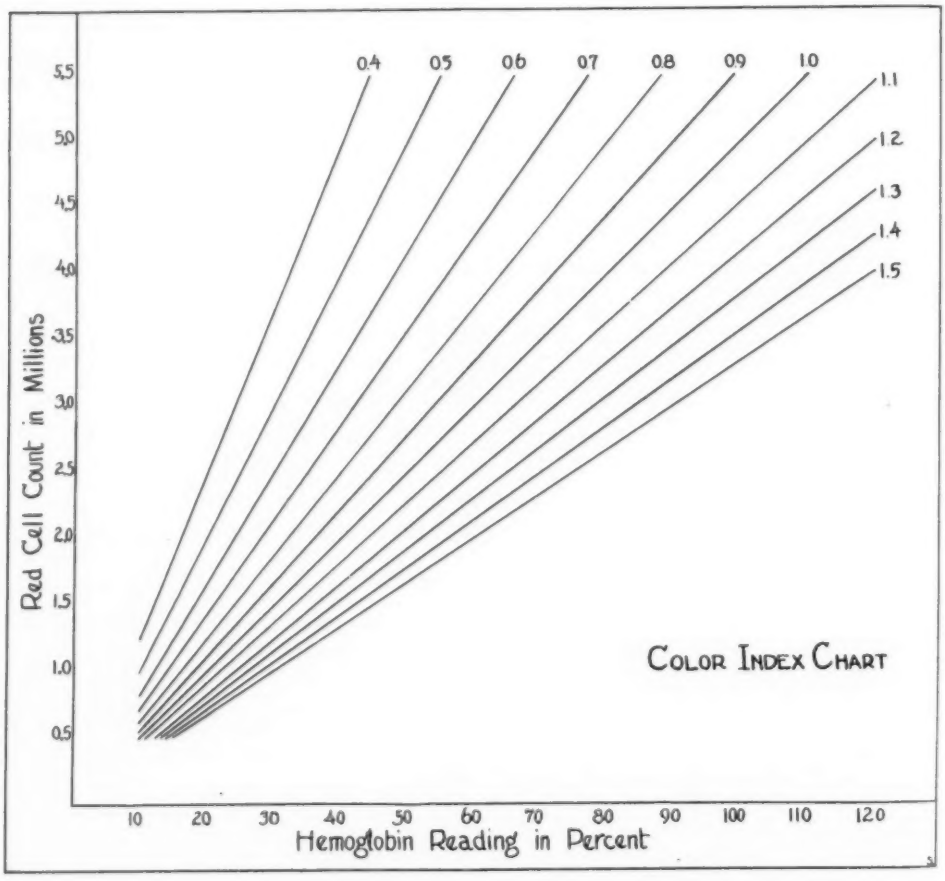
[11]Joslin, Elliott P.: The Treatment of Diabetes Mellitus, third edition, Lea and Febiger, p. 340.

[12]Bender, Theodore, and Polowe, David: Blood Density and Blood Tension during Spinal Anesthesia; unpublished data; from the Gynecological Service of the Barnert Memorial Hospital.

A CHART FOR READING THE COLOR INDEX OF BLOOD*

By Herbert Silvette, Richmond, Virginia

THE accompanying chart was devised to make the determination of the color index of blood a simple matter. This chart is based on the formula, color index equals percentage of hemoglobin divided by percentage of red blood cells. By locating the hemoglobin percentage on the abscissa and the number of red blood cells per cubic millimeter on the ordinate, the color index will be found either on one of the oblique lines, or at such a point between them that the color index can be easily and accurately estimated to 0.01.



There seems to be no such chart as the one above described in use at the present time. By means of it the color index may be read directly and the procedure should be routine in connection with red blood counts and hemoglobin estimations.

*From the Pathological Laboratory, Johnston-Willis Hospital, Richmond, Virginia. Received for publication, Jan. 24, 1927.

A SIMPLIFICATION, NOT A MODIFICATION, OF THE KOLMER TEST*

By James B. Rucker, Jr., A.B., M.D., Toledo, Ohio

SO MUCH has been dished up for the consumption of medical readers during the past five years concerning the standardization of the Wassermann test for syphilis, that it may seem rather presumptuous on my part to again stir up the same old hash and attempt to feed it to you with the same old spoon by simply mixing it together a bit more and warming it over. I know a little too well, however, from my own personal experience, that you could not be expected to swallow such a warmed over concoction and pretend to relish it.

I am frank to say that I do not intend to urge any new method for the standardization of so important a test as the Wassermann, for we have a perfectly satisfactory one already. We all are agreed by this time, that the most excellent method worked out by Kolmer and his assistants over a period of many years of painstaking effort, has given us a method far superior in its results in eliminating inaccuracies, to any which has thus far been devised.

It has been shown in thousands of tests, that its delicacy in detecting exceedingly small amounts of syphilitic antibody in the blood or spinal fluid, is as great as could be desired, without any tendency to err on the positive side. To attempt to devise a method of greater sensitivity than that set forth in the Kolmer test would be to condemn many a patient to untold anxiety concerning a disease which he never had, or if he did have, of which he is now free so far as is shown by the production of syphilitic antibodies in the tissues of the body and their circulation in the blood stream.

On the other hand, if a patient has not a syphilitic infection, the exceedingly well-balanced technic of the Kolmer test, wherein the amount of amboceptor is delicately adjusted to the complement used, and the amount of complement just as delicately adjusted to the amboceptor, immediately before each series of tests is run, the negativity of the reaction comes out strong and clear, without any equivocation, and to one who has run a sufficient number of these tests to have learned its perfect reliability, there is the supreme satisfaction of absolutely knowing that this patient's blood is free of any luetic taint. In other words, the Kolmer test picks up all the positives of whatsoever degree of which there may have been some doubt by any other test; and is especially valuable in treated cases where the physician is anxious to know what, if any, progress he is making in freeing his patient of the luetic infection; but never, throughout my experience of more than four thousand tests, has it given a positive reading when the

*Read at the Seventh Annual Session of the Ohio Society of Clinical and Laboratory Diagnosis, at Columbus, Ohio, May 12, 1927.

family and personal history, thoroughly delved into, and clinical evidence in the patient himself, presented negative findings.

If, then, it is recognized and agreed by a large majority of clinical pathologists that the Kolmer test is the most reliable complement-fixation test yet devised for the detection of the syphilitic antibody in the blood or spinal fluid, why is it, that we do not all adopt it as the standard method of performing the complement-fixation test for syphilis in our laboratories and use it to the exclusion of any other such test as a basis for reporting our results in what is still wont to be called the Wassermann test? Or, better still, why not report all our results of the complement fixation for syphilis as negative or positive (of whatever degree) Kolmer tests, for Wassermann long flourished in his day, and now deserves a well-earned rest?

Kolmer in his foreword in his articles in the *American Journal of Syphilis* and other publications, wherein he gives the technic of his standard test in the clearest detail, has struck at the root of the matter and has given us *one* answer to my question when he says that in his long search for a standard method he has evolved one which although the most accurate and reliable in its freedom from error on one side or the other, that has yet been offered, it is still more time-consuming than most of the technics at present in use, and advises those who are willing to sacrifice accuracy of result to rapidity of performance in seeking a short cut in serologic diagnosis, to scrupulously avoid his test, for such is not for them, and will lead only to disappointment.

Another reason, and the main one I think, that many clinical pathologists have not adopted Kolmer's modification as the one exclusively used in their complement-fixation tests for syphilis, is not that the result may not be reported for twenty-four hours after the test has been begun, but that the test seems to some, unreasonably complicated with the "set up" of its six tubes for each serum, the antigen control, the corpuscle control, and the reading scale for each series. This need not greatly worry one, however, inasmuch as this becomes comparatively simple when one has, after some experience, become accustomed to the various details. Nevertheless, in view of the fact that the great reliability of the Kolmer test had been so strongly impressed upon me by a great deal of personal experience with it in its original form, it occurred to me that if I could simplify the number of manipulations of the test itself, without in any way modifying its essentials, its adoption as the exclusive complement-fixation test for syphilis by the smaller hospitals and private clinical laboratories, would be greatly furthered.

Consequently, in my first attempt at simplification, I eliminated the reading scale, after having used it for some time, and having found its use added nothing to the interpretation of the reading of my results. My readings of the results of the tests themselves without the reading scale's aid were precisely the same as they were with it. I had always felt that the setting up of the scale was superfluous in the Kolmer test, inasmuch as it had seemed to me to be based upon a wrong theory of the manner by which the results were to be interpreted; because, the quantitative result as regards the *degree* of positivity does not depend upon the *degree* of fixation of complement in any individual tube dilution of the serum under examination, but upon the ability

to determine whether or not there is even the *slightest amount* of fixation in any individual tube dilution in the series. Kolmer specifically states in regard to the interpretation of the reaction, that a *very strongly positive* reaction is indicated when there is *partial* or complete fixation in the first four or all five tubes; *strongly positive,* when there is *partial* or complete fixation in the first three tubes; *moderately positive,* when there is *partial* or complete fixation in the first two tubes; *weakly positive,* when there is *partial* or complete fixation in the first tube only; and *negative* when *all* tubes show complete hemolysis. Again, in a later paper, in regard to interpretation he says: When there is *fixation of any degree* in the first four or all five tubes—and so on. Therefore from these citations, we see that in describing the manner of interpretation of the test, he dwells particularly on the words *partial or complete fixation* and *fixation in any degree.*

If, then, the *degree* of fixation in the individual tube is unimportant, according to Kolmer, why should the test be complicated by consuming additional time and labor involved in setting up a reading scale for comparison when the reading of the results of the test itself depends not at all upon the *degree* of fixation or hemolysis in the individual tube dilution, but upon the fact as to whether or not fixation has occurred in *any degree* whatsoever? Personally, I can see no good reason for its retention as a part of the Kolmer Qualitative Test, and although I discarded it some three or more years ago, I am quite satisfied that my results have been as good since that time, as they were when I was still using it This has, I feel, eliminated quite a good deal of time and labor and has considerably simplified the quantitative test.

I worked along for some time, without further elisions in the test, until one day the thought occurred to me that if the first four tubes only, or if all five tubes, show partial or complete fixation, the result is the same—*very strongly positive.* Why, then, shall I not eliminate the fifth tube?

In the several thousands of tests performed I had never secured fixation of any degree in the fifth tube when such fixation was absent in the fourth tube, although I had oftentimes secured fixation of some degree in the first four tubes, with complete hemolysis in tube five. The conclusion naturally to be drawn, was that, if fixation occurred in any degree in the first five tubes, or if it occurred only in the first four tubes, and the fifth tube showed complete hemolysis, the result was the same, *very strongly positive;* therefore the use of the fifth tube was superfluous. I am not fostering a political machine in my laboratory, so when one of my heretofore supposedly useful workers failed to show cause why he should be retained on the rolls, I dismissed him—and out went the fifth tube, with no modification, so far as I could observe, of the delicacy or accuracy of the Kolmer Quantitative Test. Of course, if fixation should ever have occurred in the fifth tube and not in the fourth, which has never happened, in my hands, I could not have reported the result as *very strongly positive,* but would have felt that my technic had been in error and that such an occurrence demanded a new set up of the serum in question and a repetition of the test.

A third simplification is that step wherein, after the second incubation has been completed and the results have been read, instead of placing the

positive sera in the ice box for three hours to settle, after which time, they are taken out and a second reading made, I have for the past two years, been placing the positives in the centrifuge and allowing them to centrifugate at high speed for three minutes, after which the second reading is made. This markedly shortens the time employed in the original test, and gives no difference in my results, so far as my observation goes. The hour's incubation in the water-bath should hemolyze all the cells that are going to be hemolyzed, provided one's reagents are properly adjusted as outlined by Kolmer, before the test itself is begun.

Unless they are, it is the height of folly to begin the main test, with the expectation that the results will be anything short of disastrous. Therefore it has seemed to me that when one means of precipitating the cells which have not been hemolyzed, is as good as another, the shorter in point of time, should be the one chosen, especially if it tends to render the test more practicable, and for that reason, more readily adopted as a Standard Quantitative Test.

As for the corpuscle control and the antigen control, it is immaterial whether one uses them or not—just as one chooses. With my known negative serum and my known positive serum controls which I always use in the set up of each series, I do not feel that they are at all necessary, and I never use them.

With more than 4000 sera I have used the Kolmer Quantitative Method simplified in the manner which has been described, and feel that while the method worked out by Kolmer has been carefully adhered to in all its essential points, I have simplified it considerably both in economy of time and labor by cutting out some of the nonessential details, so that it takes very little more time than the older Wassermann technic, with its two or three antigens, and it is far more satisfactory in its results. When I get a Kolmer, 4, 3, 2, 1, plus or a negative now, I feel that it is absolutely right, the last word has been said. Whereas, in using the old Wassermann method, I oftentimes was perplexed to determine whether my result was a 3 plus or a 2 plus or a 2 plus or a 1 plus. Now, with the Kolmer test so simplified, I am confident when I state the *degree* of positivity.

I tritrate my complement and amboceptor in the afternoon and set up my series of tests at the end of the day just before leaving my laboratory, put them in the ice box, and the next day at noon, treat them with amboceptor and sheep cells, and put them in the water-bath for an hour. Three minutes in the centrifuge for the positives completes the process, and they are ready to be reported. Thus performed, it is exceedingly practicable in my own small laboratory, and I am sure it will be in yours. Try it. In comparison, in satisfaction as to accuracy of results, there is no equal to the Kolmer Quantitative Test.

A METHOD FOR THE DETERMINATION OF THE COAGULATION TIME AND RETRACTION TIME OF THE BLOOD*

By J. W. Sooy, M.D., and Theodore S. Moise, M.D., New Haven, Conn.

THE object of this communication is to present a simple capillary-tube method for the determination of the coagulation time of the blood, which has the advantages of utilizing a minimal quantity of blood and allows a reading of the time of complete retraction of the clot. The advantages of the latter determination are too obvious to require any discussion at the present time.

A discussion of the factors concerned and the various methods of estimating the coagulation time is also unnecessary as they have been very thoroughly reviewed in papers by Hinman and Sladen (1907) and by Cohen (1911). The latter author has discussed thirty-one methods and modifications for the determination of the coagulation time of the blood.

Among these methods are ten in which the blood is drawn from a small puncture wound into capillary tubes. They are all modifications of one of the four following methods: (1) *Vierodt's:* The adhesion of fibrin threads to a white horsehair, after withdrawal from the capillary tube indicates the beginning while the completion of coagulation is indicated when the coagulum no longer adheres to the thread. (2) *Wright's:* The investigator blows through the blood-containing capillary tubes at different intervals; when the blood cannot be dislodged, it is called "clotted." Other stages are designated by the terms "liquid," or "clotting." (3) *McGowan's:* The end of the capillary tube is broken off at different intervals and the first stage of coagulation is indicated by a minute shred stretching between the broken ends. Care must be taken to keep the broken ends approximated until the reading is made. (4) *Schultz's:* This method utilizes a capillary tube four inches long blown out into a number of regular expansions forming tiny bulbs with short spaces between. The tube is filled with blood and at definite intervals a bulb is broken off and shaken in physiologic salt solution. The end point is designated as the time when the bulb remains filled with clot and only a few red blood cells drop into the solution.

All of these methods have been unsatisfactory in Cohen's hands on account of their variable readings He concludes that the most accurate is Addis' modification of Brodie and Russell's method which requires a special instrument and is too intricate and cumbersome for general use. Among the methods that he has reviewed Milian's is the simplest. This method consists in allowing a drop of blood to fall upon a glass slide, which is tipped vertically at frequent intervals. The coagulation time is read when the drop does not change its shape and maintains its convexity of outline. Cohen

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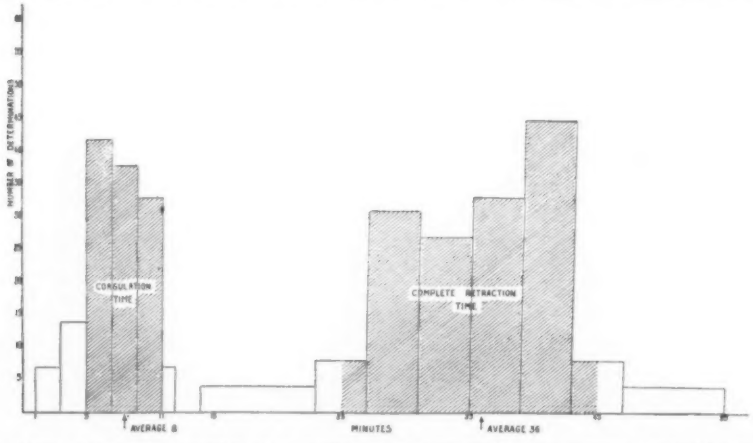
believes that his own modification of Milian's method is the best one available. This modification (Cohen's) consists of apparatus devised to prevent evaporation and maintain a constant temperature and has the disadvantage inherent in any method requiring special apparatus.

Lee and White (1913) have described a method for which they claim the advantages of simplicity and reliability. In this method 1 c.c. of blood is withdrawn by vena puncture with a small sterile glass syringe which has been rinsed with physiologic saline. The needle is removed and the blood emptied into a small clean glass tube which has also been rinsed with physiologic saline. The time at which the blood is withdrawn is accurately noted. The tube is rotated endwise every thirty seconds and the end point is read at the time at which the blood no longer flows but maintains its surface contour when the tube is inverted. Among the advantages described for this method are that the blood is obtained without contact with the skin or other tissue and that a sufficient amount of blood is obtained to study the charac-



The chart shows the results of a series of determination on normal individuals for the purpose of standardizing the method. The shaded portions indicate the time intervals within which a large majority of determinations of the coagulation time and time of complete retraction fall.

ter, color and retraction of the clot. This method has the disadvantage of requiring a vena puncture to obtain the required amount of blood.

The method to be described differs in principle from the older capillary tube methods, utilizes a minimum amount of blood, and affords the additional opportunity of studying the retractability of the resultant clot.

METHOD

Preparation of Capillary Tubes.—Glass tubes of convenient length and about 5 mm. in diameter are thoroughly cleaned, dried and carefully protected from dust. Capillary tubes about 0.3 mm. in diameter are drawn and cut into 3 cm. lengths. These tubes are measured with a micrometer and only those with an inside diameter varying between 0.2 mm. and 0.3 mm. are selected.

Drawing the Specimen of Blood.—The skin is washed with alcohol and ether, care being taken to have the area quite dry. The puncture is made of

sufficient depth to give a free flow of several drops of blood. The tube is held with forceps and filled by capillary attraction from the first drop of blood. The tube is placed on a clean glass slide and observed with low power magnification using an ocular micrometer.

Observation of the Specimen.—The time of the puncture is recorded and the following changes are observed: (1) A thin opaque line appears between the blood and the walls of the tube (average time of occurrence in normal individuals two minutes and forty-four seconds). There is a gradual appearance of a serrate outline to the marginal red blood cells and the opaque streak becomes wider (average time of occurrence in normal individuals four minutes and fifteen seconds). (2) The blood begins to retract from the wall of the capillary tube. This has been taken arbitrarily as the end point for the "coagulation time." The average time for normal individuals is eight minutes. (3) The retraction proceeds for approximately thirty-five to forty-five minutes and is best observed by adjusting the micrometer so that a mark coincides with the retracting clot margin. The change is noted at frequent intervals. The interval from the moment of puncture to the instant at which no further retraction occurs is designated the time of complete retraction. The average retraction time for normal individuals is thirty-six minutes.

DISCUSSION

The method was devised by one of us (J. W. S.) for use in experimental studies on white rats and has been utilized in the clinic in our studies on the treatment of purpura hemorrhagica by exposure to the mercury vapor-quartz lamp (Sooy and Moise, 1926). The procedure has been standardized by performing approximately 150 determinations on fifty normal individuals. The results are given in the accompanying graphic chart, which shows that a very large majority of the determinations fall between five and eleven minutes for the coagulation time and between twenty-five and forty-five minutes for the time of complete retraction. The averages for these determinations are eight and thirty-six minutes respectively. The determinations were done at temperatures ranging from 20° to 25° C.

The chief advantages in the method are the utilization of minimal quantities of blood and the opportunity it affords for observations upon the retractability of the blood clot; on the other hand, its chief weakness is that the methd requires a certain amount of practice in learning the exact end point for determination of the coagulation time.

REFERENCES

Cohen, M. S.: Arch. Int. Med., 1911, viii, 684.
Hinman, F., and Sladen, F. J.: Bull. Johns Hopkins Hosp., 1907, xviii, 298.
Lee, R. I., and White, P. D.: Am. Jour. Med. Sc., 1913, cxlv, 494.
Sooy, J. W., and Moise, T. S.: Jour. Am. Med. Assn., 1926, lxxxvii, 94.

A VARIABLE FILTER FOR THE MICROSCOPE*

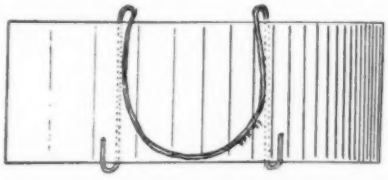
By Roy F. Feemster, M.D., D.P.H., Louisville, Ky.

SLIDES stained differently often need more or less of the red rays filtered out to obtain the best results when examining with artificial light, but very few microscopes are equipped with any kind of filtering apparatus except a blue glass disc.

The writer has tried a number of filters but the disadvantage of most of them is that the amount of blue cannot be varied. Some time ago he devised one which had this desirable feature. It has proved exceedingly satisfactory and has the added advantage of being very simply made.

The filter consists simply of a strip of photographic celluloid film, about 1¼ inches wide by 3½ to 4 inches long, which is stained heavily with methylene blue at one end and shades off to almost colorless at the other end. This strip is slipped under the ring which usually holds the filter disc to the substage condenser. It can then be moved to the right or left as desired, thereby giving varying amounts of blue.

Many microscopes, instead of having the hinged ring to hold the disc, have a slot into which it is slipped. In this case a wire attachment, like that shown in the accompanying illustration, must be made to hold the filter under the



substage condenser. The curved portion of the wire is slipped into the slot and the filter can be moved to the right or left.

I obtain large sized films from the x-ray laboratory and remove all of the gelatine emulsion by treating with hot water. The filter is best colored when making several at a time, that is, cut off a strip of the film about 3½ inches wide, lay it on the table and wet the surface with a damp cloth. Then take a small brush and apply methylene blue dissolved in wood alcohol, beginning at one side and gradually working toward the other, carrying the brush from end to end in long sweeping strokes. Dip the brush repeatedly into the methylene blue solution and repeat the operation. The wood alcohol in high strength softens or dissolves the surface layer of the celluloid and the methylene blue is precipitated into the film. The gradual decrease in the strength of the alcohol, as diluted by the water applied in the beginning, insures a gradually diminished intensity of color. After the strip has been stained properly it is cut into smaller strips about 1¼ inches wide. The methylene blue gradually fades, so all except the filter in use should be put away in the dark.

This filter makes it possible to have a high degree of illumination without the usual eye strain. Students in pathology here who have been given these filters are vastly pleased with the added comfort and the ease in distinguishing structures.

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CEDAR OIL AS AN AID IN FINDING PARASITIC OVA IN FECES*

By Gordon E. Hein, M.D., San Francisco, Calif.

FOR several years, at the San Francisco Hospital, we have been using a method in searching stool specimens for parasitic ova which we feel deserves a more widespread application.

It depends upon the property of cedar oil in clarifying smears of dried

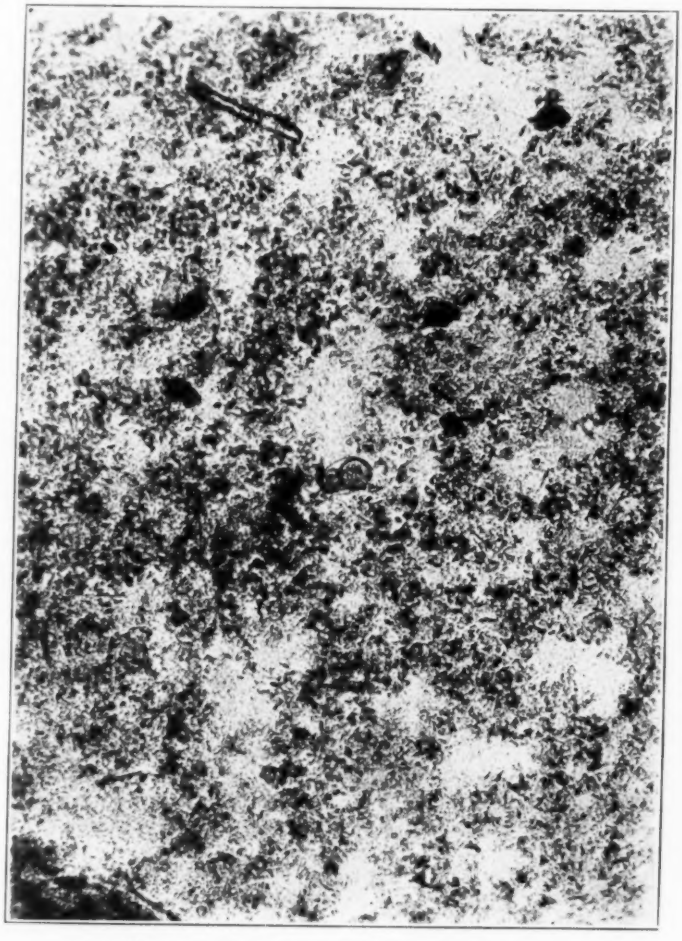

Fig. 1.—Fresh feces showing a single egg.

feces so that very thick smears may be utilized in looking for ova, with greatly increased probability of finding them.

An extremely thick smear of the suspected feces is made upon a slide and allowed to dry at room temperature. The thickness of the smear is

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about from five to ten times as heavy as ordinarily would be used, and a few trials will show the approximate thickness necessary.

Cedar oil is dropped upon the field and covered with a cover glass. The feces are rendered transparent and ova are greatly accentuated by the clear background.

The slides may be kept for some time and we have ova of trichocephalus trichiura which are unchanged after three years.

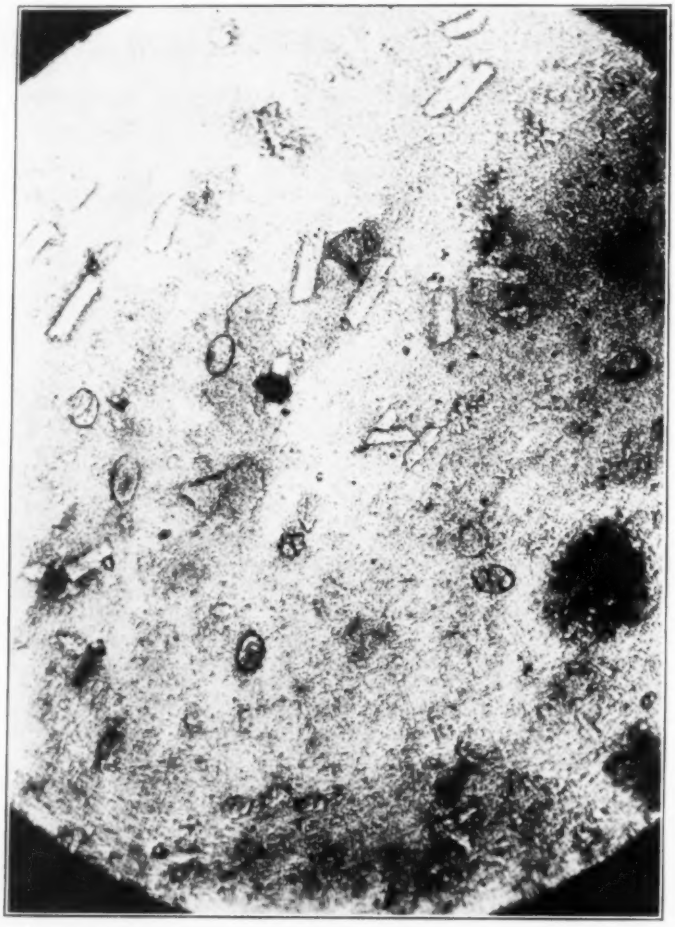

Fig. 2.—Dried smear clarified by cedar oil. Background much clearer in spite of thickness of smear.

Fig. 1 shows a fresh smear of feces in which a single hookworm egg was found after prolonged search. A thick smear clarified with cedar oil showed six ova to one field. (Fig. 2.)

By this method we have found ova in feces without difficulty where repeated search of fresh smears without concentration methods failed to show them and, without doubt it could be used to supplement other concentration methods such as centrifuging or the brine flotation method of Kofoid and Barber. The simplicity and ease with which ova are revealed seems to us to advocate the method strongly.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

LYMPHOBLASTOMA: Lymphoblastoma: Aspects Concerning Abdominal Lesions, Especially Their Production of Early Symptoms, Minot, G. R., and Isaacs, R. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 157.

Twenty-five per cent of 477 patients (401 dead, 76 living) with lymphoblastoma, exclusive of lymphatic leucemia, had as their initial symptom one referable to an abdominal lesion. Twenty per cent of the deceased without such initial symptoms had symptoms referable to abdominal disease early in the course of their illness. Late in the disease such symptoms occurred with very great frequency.

Symptoms due to lymphoblastoma within the abdomen are protean and may simulate many kinds of disorders. They often lead to an incorrect diagnosis, but need not necessarily do so if the condition be recalled and all facts evaluated properly.

Abdominal and back pain, often intense; pains in the legs and various gastrointestinal symptoms may be prominent early ones. Genitourinary symptoms, jaundice, signs of invasion of the pancreas, adrenals, and spinal cord are apt to appear late. Fever and skin manifestations may occur early and even be initial when the chief lesion is in the abdomen. Pregnancy may run its normal course in patients with lmphoblastoma, and is not unusual.

In but 27 per cent of the 119 cases with initial symptoms referable to abdominal lesions, was there enlargement of lymph nodes in the groins early, and at this time only 23 per cent had enlargement of other external nodes.

Cases of this sort occur more often in the later than the earlier years of life; even so, cases with early, but not initial symptoms referable to abdominal lymphoblastoma occur particularly in the third decade of life.

After the appearance of symptoms referable to an abdominal lesion, the prognosis for a long duration of life is worse than when such manifestations are absent.

Irradiation of abdominal lesions can alleviate markedly these patients' symptoms. It has not prolonged definitely, on the average, the duration of disease but probably occasionally can do so. Likewise, rarely the duration of a case may be extended by a radical surgical operation.

PERITONITIS, MERCUROCHROME IN: The Treatment of Experimental Peritonitis by Mercurochrome—220 Soluble, Wiles, A. M., and Haskell, C. C. Arch. of Surg., May, 1926, xii, 1080.

A series of experiments upon dogs in which experimental peritonitis was produced by incision of the large bowel. The animals were treated by the intravenous injection of 5 mg. of mercurochrome in 1 per cent solution per kilogram body weight. In every instance in which the animal survived more than twenty-four hours, this dose was repeated daily until death or apparent recovery was manifest.

While slightly beneficial results were noted in twenty-five animals no spectacular effect was seen and, in the endeavor to bring this out, a further series received, in addition to the mercurochrome, 10 c.c. of 25 per cent glucose per kilogram.

The dextrose-treated animals showed a higher mortality than those treated with mercurochrome alone, possibly because the hypertonic dextrose solution hastened absorption from the peritoneal cavity.

The authors are not enthusiastic about the value of mercurochrome in peritonitis, recognizing, however, that peritonitis is a toxemia as well as a bacteriemia.

RHEUMATIC FEVER: Rheumatic Fever. An Analytic Study of Three Hundred and Ninety-three Cases of Rheumatic Fever and Eighty-nine Cases of Chorea, Mackie, T. T. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 199.

Rheumatic fever in approximately 70 per cent of all cases, irrespective of age, presents itself as a chronic disease, characterized by periods of recurrence of the acute features—fever, arthritis and leucocytosis. The age of the patient is a highly important factor in the prognosis. This is evident from the fact that under the age of fifteen years is found the highest incidence of first attacks of cardiac involvement and of liability to recurrence. Serious cardiac involvement occurs in 68 per cent of cases irrespective of age. Between the ages of ten and fifteen years approximately 78.2 per cent of all cases present evidence of this complication in the first attack, and only after the age of twenty-five years does the incidence of heart disease fall below 50 per cent in the initial attack of rheumatic fever.

That focal infection plays a rôle in the etiology seems apparent from a comparison of its occurrence in the rheumatic cases, with a series of 400 nonrheumatic controls. In the former group it was found in 80 per cent of individuals as against 66 per cent in the latter group. Tonsillar infection was found to be more than twice as prevalent in the rheumatic fever cases as in 400 nonrheumatic controls. The complete removal of the tonsils when evidence of infection is present, together with appropriate treatment of other foci of infection seems to reduce but not to remove the incidence of recurrences of rheumatic fever.

The expected incidence of recurrences of all age groups was found to be 71 per cent. In patients above the age of twenty years, at the time of the first attack of rheumatic fever, it was found to be 58.6 per cent, while below the age of twenty the incidence rate was 78.2 per cent. In a general way, the younger the patient at the time of the first attack of rheumatism, the greater the probability of recurrences; 93 per cent of all cases having the first attack between the fifth and tenth year have recurrences of the acute condition.

Only 57 per cent of the first recurrences were found to develop within a period of four years following the first attack of rheumatic fever. This would seem to be a very important factor in analyzing the true worth of any therapeutic or prophylactic attack upon the problem of rheumatism.

DIABETES: Constitutional and Hereditary Traits in Diabetes, Borach, J. J. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 243.

A correlation of observations upon 350 cases.

Nutritional History: Recorded at four periods:

A. At maturity—21 years: 100 cases; weight normal.

B. Maximum weight and age at which attained: between the age of 21 and the time diabetes was discovered, the average weight increase was 59.5 pounds.

C. The time when weight began to diminish.

D. The weight at the beginning of treatment.

For the series, 90 per cent were obese.

Dietetic History: A history of overeating was found in only 20 per cent.

Height and Weight: No characteristic deviation from normal, other than excessive fat deposits, was encountered.

Complexion: Light complexion is very characteristic and an element in the composite picture of the typical case of diabetes.

Hirsuties and Skin: The typical diabetic is light complexioned, has a scant covering of hair over the body, and the skin is abnormally smooth.

The thyroid gland is frequently involved and skin lesions, such as papillomas, angiomas, xanthomatous placques, and diffuse brownish pigmentations are not infrequent.

Hereditary Tendency: A definite familial history was encountered sixty-eight times.

If it cannot be said that an individual having these stigmata is predestined to become diabetic, these observations do justify us in saying that in diabetes they occur with striking coincidence. We look upon the stature, obesity, coloring, lesions of the skin, endocrine disturbances, family history of diabetes and the other findings to which we have referred as in-

dicating constitutional disturbances, which make an individual diabetic. They seem intimately concerned in the cause and the effect of the disease. An individual who presents these findings, is the kind of an individual who develops diabetes.

CHOLERA INFANTUM: Upper Respiratory Infection as a Cause of Cholera Infantum, Jeans, P. C., and Floyd, M. L. Jour. Am. Med. Assn., July 24, 1926, lxxxvii, 220.

Observations of the authors lead them to believe that there is a relationship between upper respiratory infection and a clinical picture corresponding to what has been described under the term cholera infantum. In recent years all patients presenting this clinical picture who have come under their observation have had either mastoiditis or paranasal sinusitis or both as the apparent underlying cause of their disturbance. The infection is seldom obvious, while the gastrointestinal symptoms are usually prominent. The establishment of adequate drainage from the site of infection brings about prompt and complete recovery.

INSULIN: Blood-Sugar Content and Insulin Treatment of Dermatoses, Simon, F. Arch. f. Verdauungskr., 1926, xxxvii, 363.

According to the author, a marked increase in blood-sugar value occurs in psoriasis and in furunculosis. In cases of psoriasis higher values are said to be noted in males than in females, while in furunculosis the increase is declared to vary within somewhat narrow limits.

Blood-sugar value is said to exhibit an increase in the majority of cases of eczema, and to vary within wider limits. Highest values were encountered in relapses and in chronic cases, and relatively high values in eczema in diabetes mellitus.

Insulin treatment of these dermatoses, which are accompanied by increase in blood sugar value, is declared to lead first to a temporary increase and later to a decrease in blood-sugar value. Thus, it is pointed out, pruritus is prevented and predisposition of the skin to injuries due to activity of bacteria is diminished.

Simon is of the opinion that insulin may through its increase in the alkalinity of the blood exert a favorable effect upon many diseases of the skin.

BILIRUBIN: The Formation of Bilirubin, Mann, F. C., Sheard, C., and Ballman, J. L. Minn. Med., May, 1926, 227.

The investigations of the authors lead them to conclude that bilirubin is formed from hemoglobin and that hematin appears as an intermediate product in the formation of bilirubin from hemoglobin.

MEASLES: Measles Prophylaxis: Use of Blood from Convalescents in a School Epidemic, Townsend, J. H. Boston Med. and Surg. Jour., May 13, 1926, cxciv, No. 19, p. 869.

The use of prophylactic measures in an epidemic of 63 cases of measles in a boarding school of 400 boys is described.

In a dosage of 20 c.c. the blood from an adult who had measles twenty years previously seemed to have no effect either in preventing or modifying the disease.

Blood from convalescents in a dosage of 9 c.c. of whole blood (5-5½ c.c. serum) had little or no effect in preventing infection, but influenced markedly the course of the disease when it was given before the end of the first week of the incubation period.

Thirty-two cases who received convalescent blood at least eight days before the development of the rash showed an average duration of the febrile period of 3.66 days, whereas 21 boys who received no inoculation showed an average duration of the febrile period of 6.45 days.

The average maximum temperature of the 32 who received convalescent blood at least eight days before the development of the rash was 102.5° F., whereas the average maximum temperature of the 21 who received no inoculation was 103.5° F.

The average stay in the infirmary of the 32 inoculated boys was 9.1 days, whereas the average stay of the control group was 13.0 days.

No complications whatever occurred in the inoculated group of 32, whereas in the control group of 21 there was one case of bronchopneumonia, one of otitis media, one of frontal sinusitis, and one of external otitis.

The mild character of the disease in many of the boys who received inoculations was very striking.

The inoculations had no ill effects whatever.

Beneficial effects were obtained whether the blood was administered as late as 6 days after exposure or as early as twelve days before the probable date of infection.

THYROID DISEASE: Calcemia and Glycemia in Thyroid Diseases with Increased Basal Metabolism, Waldorp, C. P., and Trelles, R. A. Rev. Soc. Argentina de Biol., December, 1925, i, 762.

The following conclusions are drawn from a study of twenty-six cases.

1. Hypocalcemia occurred in all cases with a basal metabolism above normal.
2. There was no absolute parallelism between the calcemia and the basal metabolism.
3. In half of eighteen cases the blood sugar was increased.

HEMOPHILIA: Blood Clotting Studies in Hemophilia, Mills, C. A. Am. Jour. Physiol., May, 1926, lxxvi, No. 3, p. 632.

Very fresh serum, obtained by clotting hemophiliac plasma with tissue fibrinogen, is found to contain a rich supply of active thrombin, but no prothrombin capable of activation of cephalin.

This thrombin very rapidly disappears from such serum, and at no time in the aging process can any new thrombin be produced by cephalin such as we see in normal serum.

Cephalin in one case actually delays, and in the other case only very slightly accelerates the clotting of recalcified hemophiliac citrate plasma. This together with its inability to act on hemophiliac serum, leads us to believe that the fault lies in some fashion in the prothrombin factor.

There is found no increase in the antithrombin of hemophiliac blood or serum.

Protein sensitization and local skin reaction in a hemophiliac generates a normally reacting prothrombin in the blood and increasing the cephalin effect on the plasma and serum.

Tissue fibrinogen clots such blood equally well whether normal prothrombin be present or absent, confirming our views as to the two independent clotting processes.

Witte peptone preserves the thrombin of hemophiliac serum and enables cephalin to exert its characteristic action.

DIABETES: Necropsy Findings in Diabetes, Wilder, R. M. Southern Med. Jour., April, 1926, xix, No. 4, p. 241.

An analysis is presented of the pathologic conditions found in a group of eighty-one fatal cases of diabetes. Diabetes was solely and directly responsible for death in ten cases, death in the remainder being due to degenerative complications or to the consequences of operations. Necropsy was performed in fifty-eight cases.

Gallstones were found in sixteen of the fifty-eight cases at necropsy. Pancreatic stones were found in three cases. Sclerosis of the kidneys occurred in fourteen instances, and chronic diffuse nephritis in four.

Arteriosclerosis of considerable degree occurred in nearly all cases when the age of the patient exceeded forty years.

Gangrene accounted for fourteen of the eighty-one deaths and was associated with a high degree of coronary sclerosis and myocardial damage in 75 per cent of cases in which the heart was examined at necropsy.

A very high incidence of advanced coronary sclerosis was encountered in the series as a whole (seventeen cases among fifty-eight), associated usually with marked fibrosis of the myocardium.

In four cases exophthalmic goiter and diabetes were combined. In two of them the pancreas revealed little or no anatomic abnormality.

Hydropic degeneration was not recognized but marked fatty changes in the islands occurred in eleven cases.

In several cases, representing the most severe and intensive instances of diabetes in the series, the pancreatic lesions were trivial. On the other hand, severe pancreatic lesions were frequently found in cases of relatively mild diabetes in which death was due to degenerative complications.

A parallelism between the degree of parenchymatous changes and the intensity of the clinical symptoms of diabetes does not exist. The explanation of the cause of diabetes must involve considerations such as heredity of predisposition and ability of cells other than those of the pancreas to elaborate insulin.

SMALLPOX: The Blood in Smallpox During a Recent Epidemic, Ikeda, K. Arch. Int. Med., May, 1926, xxxvii, 660.

An analysis of 250 examinations of 200 cases.

The blood of smallpox shows characteristic findings which, if properly interpreted, are of definite diagnostic and prognostic value.

The earlier the rise of the platelets, the sooner the approach of the desiccation period, prognosticating a shorter course of the disease.

A definite leucopenia during the maculopapular stages indicates, as a rule, a mild discrete form. A progressive leucocytosis with an early high polynucleosis predicts a severe form. The higher the values, the more probable the fatal outcome.

The early appearance of normoblasts, basophilic stippling and polychromatophilia, without evident anemia, is an unfavorable sign. It invariably means the purpuric form of smallpox.

Condensation and fragmentation of mature leucocytes are found only in the purpuric form of smallpox. They appear comparatively early in the primary type of purpuric smallpox and are usually accompanied by pathologic normoblasts, basophilic stippling and polychromatophilia, without visible anemia.

A rapidly progressive, absolute lymphocytosis is a constant characteristic of purpuric smallpox.

Scarlatinal and other exanthems, infectious purpura and toxic rash with petechiae, etc., can be definitely differentiated from the purpuric form of smallpox during its erythematous stage by these blood findings.

THYROID DISEASE: Kottman's Reaction, Basal Metabolism, and Biologic Tests, Etienne, G., Richard, G., Karall, E., and Claude, F. Compt. rend. Soc. de biol., March 19, 1926, xliv, 667.

A study conducted upon twelve cases in which the results of the Kottman test were correlated with other examinations. The authors believe that the Kottman reaction is unreliable and gives such uncertain results in thyroid disease that it cannot be safely used as a means of diagnosis.

WASSERMANN REACTION: Wassermann and Flocculation Reaction in Luetic Milk, Hackman, P. Münch. Med. Wchnschr., May 7, 1926, lxxiii, 774.

A report of examinations made in thirty cases.

The milk was first shaken with ether, then centrifuged at high speed, and tests conducted upon the clear liquid under the fat layer. The author states that the ether extraction did not interfere and that anticomplementary reactions were definitely decreased.

Parallel tests were also made upon Berkefield filtrates of milk.

In twenty-four nursing women the Wassermann reaction was positive in both blood and milk later becoming negative. Ten were treated in pregnancy, five in childbed, four in pregnancy and childbed.

In five cases the blood was negative and the milk positive. In three treated cases both blood and milk were negative.

Rusca's observation, that the milk becomes negative sooner than the serum when the patient is treated, was not confirmed.

Slight oscillations in the strength of the reaction even on the same day were noted.

Nonspecific fixation in nonluetic milk was occasionally encountered.

In luetic milk positive reactions were encountered in as little as 0.025 c.c.

The flocculation reactions, (Meinecke's, Dold's, and Sachs-Georgi), were generally variable and much weaker, the first being the easiest to read.

DIAGNOSTIC CASE: A Useful Diagnostic Aid Case, Piercy, H. D. Jour. Am. Med. Assn., May 29, 1926, lxxxvi, 1689.

Piercy describes and illustrates a diagnostic aid case to be carried in the physician's bag.

The case, of German silver, is hinged on one side and pipettes for making blood counts; glass cover slips for blood smears; glass slides for blood or pus smears; a blood agar slant with sterile swab; a sterile test tube for blood or other fluid; hypodermic vials, containing, in one calcium oxylate, and, in the other, sealed capillary tubes for formaldehyde; four 8 c.c. glass vials containing, respectively, diluting fluid for white and for red cells, alcohol and ether; several needles for venipuncture and lumbar puncture, and a blood lancet.

The cover slips are contained in a small square box in the upper right hand corner. The culture tube made by the Digestive Ferments Company of Detroit, happens to be just the proper size to fit into this case. The spring clips securely sealing the ends of the blood pipettes are of obviously simple construction and should be obtainable from any supply house. The articles are secured in place by nickel plated spring clips of phosphor bronze, soldered to the top and bottom of the case.

LABORATORY TECHNIC

SYPHILIS: A Study of Testes from Syphilitic Patients, Saleeby, E. R.: Am. Jour. Syph., April, 1926, x, 2.

The testes from forty syphilitic human subjects were studied grossly and histologically. In addition to the varying degrees of fibrosis there were, grossly, no characteristic changes. Microscopically, twenty-three cases, or 57.5 per cent, showed pathologic changes commonly attributed to Spirocheta pallida infection. In seventeen of the twenty-three cases, the Wassermann reaction was positive; in two, negative, and in four, not made. The Levaditi preparations were negative for the organism in all the cases except in a seven-month-old fetus, where the spirochetes were demonstrated in large number. Eight rabbits were injected intratesticularly, with emulsions from eight cases and observed for four months. None of the rabbits developed syphilomas. The findings in this study tend to show the frequency of the disease in the testicles and the difficulty of demonstrating the organism in the tissues with our present methods and present knowledge of its morphology.

SYPHILIS: Twenty-five Years of Congenital Syphilis in Boston, Sylvester, P. H. Jour. Am. Med. Assn., July 31, 1926, lxxxvii, 298.

The fetal and infantile mortality and morbidity from congenital syphilis should make it a subject of importance in our work.

The Wassermann reaction, the development of arsenical therapy, the specially organized clinic and the social service have combined to effect a satisfactory reduction in mortality and morbidity, provided treatment is instituted during the critical period of from five weeks to two months.

The foregoing factors have had very little effect on cases appearing for treatment much before or much after this period.

More effective treatment for the very early case must be developed, or its prevention must be obtained through treatment of pregnant mothers.

Sulpharsphenamine, intramuscularly, appears to be nearly as effective as neoarphenamine, intravenously, in causing disappearance of lesions and serologic reversals in early cases.

It appears to be as effective in causing the lesions to disappear in late cases and more effective in reversing the serum.

It is possible that a definite reduction in the incidence of congenital syphilis may be brought about through educational and legal measures.

ENCEPHALITIS: Studies on the Etiology of Epidemic Encephalitis, Evans, A. C., and Freeman, W. Pub. Health Rep., June, 1926, xli, No. 23, p. 1095.

A pleomorphic streptococcus, highly virulent for rabbits when inoculated intracerebrally, was obtained from the nasal washings, heart blood, and mesencephalon of a case of epidemic encephalitis.

In so far as the comparative tests have been made, this streptococcus agreed with the streptococci obtained from cases of epidemic encephalitis by Von Wiesner and by Rosenow. Apparently several other investigators have cultivated the same organism in their studies of the disease.

When inoculated intravenously into rabbits the streptococcus shows a tendency to elective localization in the brain.

In rabbits and in monkeys it produces nervous symptoms which in some cases simulate the disease in man.

Rabbits inoculated with this streptococcus show no inflammatory lesions outside of the central nervous system. The meninges are heavily infiltrated with lymphocytes and leucocytes, the inflammation spreads to the cerebral substance by direct extension and along the small vessels, penetrating into the brain. There are severe parenchymatous degenerative changes in the nervous tissue and reaction of the neuroglia. The sheaths of the blood vessels are found infiltrated by lymphocytes. The reaction is sometimes most marked in the mesencephalon.

In monkeys there is noted a greater tendency toward leucocytic reaction, and in two instances large areas of hemorrhagic inflammation in the basal ganglia were noted.

POLIOMYELITIS: A Skin Reaction In Poliomyelitis, Rosenow, E. C. Jour. Infect. Dis., June, 1926, xxxviii, No. 6, p. 529.

Rosenow injected 0.1 c.c. of a 1:100 dilution of a killed culture of a pleomorphic streptococcus.

The absence of marked reactions in persons fully recovered from poliomyelitis and who are known to be immune; the incidence of positive reactions inversely according to age, corresponding in general to the age incidence of poliomyelitis; the strongly positive reactions during the acute stage of the disease, and the negative reaction during convalescence, are considered as presumptive evidence that the test is a measure of susceptibility to poliomyelitis.

Numerous questions regarding the nature of the reaction have not yet been worked out. The immune serum prepared from horses with the pleomorphic streptococcus, and used with apparent benefit in the treatment of the early stages of poliomyelitis, however, has a marked neutralizing power over the toxin, as determined by the skin reaction.

COLDS, BACTERIOLOGY OF: Observations of the Normal Bacterial Flora of Nose and Throat with Variations Occurring During Colds, Shibley, G. S., Hanger, F. M., and Dochez, A. R. Jour. Exper. Med., March, 1926, xliii, No. 3, p. 415.

The normal bacterial flora of the nose and throat of thirteen individuals has been studied over periods ranging from five to nine months.

Observations have been made of qualitative and quantitative changes in the flora occurring in the course of colds and of throat infections appearing in the group.

The normal basic nasal flora includes Staphylococcus albus, diphtheroids, and for certain individuals Staphylococcus aureus and citreus. Occasional transient bacteria are Gram-negative cocci and nonhemolytic streptococci.

The normal basic throat flora includes Gram-negative cocci, nonhemolytic streptococci,

and for certain individuals "large Gram-positive cocci," B. influenzae, Bacillus "X," and diphtheroids. Transient organisms are Staphylococcus albus, hemolytic streptococci, Staphylococcus aureus and citreus, and pneumococci.

No bacteria were found in early cold cultures to which a causative rôle could be assigned.

In the course of colds the basic flora of the nose was often scanty in the early stages. The throat showed reduction of prominence or alterations in predominance of the basic flora.

Certain organisms were prominent in colds, usually, as late or secondary invaders, these included Staphylococcus aureus, hemolytic streptococcus, and B. influenzae.

There was a striking increase in the incidence of hemolytic streptococci in throat infections.

WASSERMANN REACTION: Concerning the Reactivation of the Wassermann Reaction, Pinard, M. Bull. soc. med. de hop. Paris, May 13, 1926, xlii, 724.

Pinard cites a case illustrating the fact that a latent syphilis with negative serology may become positive after a reactivation and reiterates the varied factors which may be responsible for such reactivation such as protein shock or trauma, pregnancy, acute infections, or acute exacerbations of a chronic disease.

He emphasizes that properly conducted tests are highly reliable.

POLICMYELITIS: Further Studies of the Poliomyelitis Precipitin Reaction, Rosenow, E. C. Jour. Infect. Dis., June, 1926, xxxviii, No. 6, p. 532.

The technic was essentially the same as that used in 1924. It was made as uniform as possible throughout the study. The swabbings were made from the nasopharynx in the same manner, gross contamination from the tongue being avoided. Readings were made under the same conditions of illumination in a darkened room, and in order not to be biased in recording findings, were often made without knowing at the time, the source of the extracts. In many instances tests on duplicate swabbings were made at the same time and repeat swabbings at short intervals. At least two different preparations of the poliomyelitis antistreptococcus serums and four control serums were used throughout the study. In some instances the precipitating power of the serum of convalescent human beings and monkeys was also tested. Blood agar platings of suspensions of the swabbings were made in many instances, both during and following the epidemic.

The results of the precipitin reaction with immune horse serums and extracts of nasopharyngeal swabbings in community and institutional outbreaks of poliomyelitis, proved positive in nearly all frank and abortive cases at the time of the attack, in a high percentage of normal contacts and in persons not exposed to the disease at the time when cases occurred. It proved negative in nearly all of the cases in from two to three weeks after the acute attack had subsided and in normal persons soon after the epidemic had disappeared. In one epidemic, the incidence of positive reactions generally was found low shortly before the occurrence of the first case, high during the period of the epidemic and again low after the epidemic had subsided. The increase in positive reactions as cases of poliomyelitis developed and the decrease as poliomyelitis disappeared occurred rapidly and seemingly independently of exposure to the disease, in isolated households in the country as well as in the urban populations. Persons who were negative to the precipitin reaction on entrance into the epidemic zone soon became positive and reactions resembling abortive attacks of poliomyelitis were common in children. The number of positive reactions in persons who came to the Mayo Clinic from widely separated communities was relatively high during the latter part of August when poliomyelitis was generally prevalent and much lower during the latter part of October after poliomyelitis had largely disappeared. After the epidemic in Rochester had subsided and the precipitin reaction in the population had become largely negative, cases occurred south of Rochester where the number of a positive precipitin reaction was high.

In certain instances poliomyelitis occurred without exposure within from five to twelve days after the presence of the streptococcus was demonstrated in the throat. Repeated swabbings showed that the carrier state lasts usually from one to three weeks in normal persons. Immunity to poliomyelitis and the occurrence of the organism in the throat did not run parallel. The positive reactions during epidemics in adults, who are relatively immune, and in children, who are relatively susceptible, were found nearly equally high, and persons who had had poliomyelitis became carriers of the streptococcus during epidemics quite like persons who had not had the disease.

LEPROSY: Notes on the Pathology of Leprosy, Wade, W. H. Jour. Philippine Islands Med. Assn., Feb., 1926, vi, 37.

Wade thus summarizes the views of the Culion leper laboratory:

The lepra cell predominates in the typical leproma but the bacilli are also to be found scattered and massed in tissue spaces and in capillaries. Some of these free-lying bacillary masses may have originated in cells which have died and disappeared.

Other leucocytes occupy no essential part of the cytologic picture of leprosy, though lymphoid cells are often present.

No particular study of the blood count seems to have been made but there seems to be a tendency to higher lymphocyte percentages and to exaggeration of leucocytosis in complicating infections.

Anemia is common in advanced cases and becomes marked in protracted lepra reaction.

Lepra bacilli have not as yet been found in the sputum in numbers sufficient to suggest pulmonary involvement, when found they apparently come from the upper respiratory passage.

The lepra bacillus is extremely refractory to cultivation or to transplantation to experimental animals.

No specific immunologic test has as yet been found. In the ordinary phases of leprosy, with a carefully adjusted technic, positive Wassermann reactions are not obtained unless there is complicating syphilis or yaws; a certain number of lepra reactions may give weakly positive results, however.

Lepra serums give positive reactions with various flocculation tests.

The red cell sedimentation test is frequently markedly positive.

Determinations of the albumin-globulin ratio by viscometry and refractometry indicate that there is often a marked globulin increase.

CEREBROSPINAL FLUID: Modifications in the Cerebrospinal Fluid in the Course of Serum Reactions, de Lavergne, V., and Abel, E. B. et M. Soc. Med. Hosp., Paris, March 19, 1926, l, 488.

Spinal fluid examinations in twenty subjects presenting an urticaria following the injection of immune serum indicated that the fluid is not entirely normal during the course of serum reactions.

The change, though slight, is distinct and evidenced by hyperglycorrhachia, lymphocytosis and the presence of some polynuclears, and sometimes merely by a hypertension. Albumin is not increased.

At the beginning of the eruption there is a normal or subnormal cytology followed by the changes above noted, the fluid again returning to normal at the end of the eruption.

The changes correspond to certain clinical symptoms such as headache, etc., which may be interpreted as the expression of a moderate meningeal reaction resulting from sympathetic disequilibrium.

SYPHILIS: The Vernes Flocculation Test for Syphilis, Baylis, A. B., Sheplar, A. E., and MacNeal, W. J. Arch. Dermat. and Syph., August, 1925, xii, 242.

A minute, detailed account of the preparation of the reagent, "perethynol," and of the technic of the test is given in this paper which should be consulted for details too lengthy for abstraction.

In this flocculation test, reported in terms of flocculation in hundredths of milligrams per cubic centimeter, 98 per cent of nonsyphilities give readings of 0.02 mg. or less.

A series of 1,000 comparative Vernes and Wassermann tests are reported from which the following conclusions are formulated:

The authors are not inclined to agree with Vernes in discarding the Wassermann test. Certainly, in the United States, the highly sensitive antigen of Kolmer and the use of low temperature for the preliminary stage of the reaction have added to the precision of this test so that it must now be regarded as highly specific and indispensable in the diagnosis and treatment of syphilis. On the other hand, the results of this test require confirmation by other evidence, especially clinical evidence. The serologic test of Vernes presents an additional laboratory check.

The Vernes test is of special value because it avoids the hemolytic system altogether, because its results are read in numerical values directly, and because it frequently conflicts with the Wassermann results in latent and treated syphilis, thus placing the physician on his guard against too slavish acceptance of either serologic result.

When employed to test successive specimens from the same patient, in accordance with the directions of Vernes, this flocculation test is able to give more precise information concerning pathologic variations in the blood than can be obtained by other methods.

The Vernes flocculation test should be regularly employed in conjunction with the Wassermann test as a help in diagnosis and treatment of syphilis.

The result of a single Vernes test is only suggestive unless the reading is high, 0.02 or above.

Repeated tests at definite intervals giving essentially the same reading in spite of provocative treatment speak against a diagnosis of syphilis, even though the reading itself be high.

On the other hand, a relatively low reading which changes appreciably on repeated tests speaks for positive diagnosis.

Conflicts between the results of the Wassermann and of the Vernes tests are especially valuable, as at once a more complete review of all the evidence and the serologic examination of additional specimens is demanded.

MERCURIALS: Mercurials: A Proposed Method of Laboratory Evaluation and Classification, Peterson, J. B. Jour. Am. Med. Assn., July 24, 1926, lxxxvii, 223.

Briefly the method consists in determining the smallest quantity of drug that will prevent the formation of gas in a yeast-sugar mixture of definite strength during a period of one hour.

The actual test solutions were made by mixing 2 c.c. of a 50 per cent sucrose solution with the desired amount of the drug and sufficient water to make the volume 8 c.c. Finally, a 2 c.c. portion of 20 per cent yeast suspension (Fleischmann's Yeast being used) was added; the whole was shaken and poured into a test tube 10 cm. long and 1 cm. in diameter. A test tube 15 cm. long and 2 cm. in diameter was slipped over the open end of the smaller tube and the whole quickly inverted. The linear distance from the end of the smaller tube to the surface of the liquid was carefully measured. After the tube had been kept at exactly 38° C. for one hour, this distance was remeasured. The tests of quantitative importance were those containing the smallest quantity of drug that yielded practically no carbon dioxide.

GLYCOSURIA: A New Table for Lactose (Milk or Urine) and Glucose (Blood or Urine) Calculation, with Notes on Their Estimation, Haskins, H. D. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 256.

The author has previously described (JOUR. LAB. AND CLIN. MED., 1923, viii, 747) a simplified Shaffer-Hartman method which, in the present paper, is again described at great length and applied to the determination of lactose in milk and urine as well as to glucose in blood or urine.

An extensive table is appended from which readings may be made directly without calculation.

The paper cannot be abstracted without almost total transcription and should be consulted in the original.

CEREBROSPINAL FLUID: Studies on the Quantitative Estimation of the Total Protein Content in Cerebrospinal Fluid, Young, G. A., and Bennett, A. E. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 249.

The following method has been used in over 600 determinations by the authors and by other workers also with perfect satisfaction.

Two c.c. cerebrospinal fluid are measured into an ordinary graduated centrifuge tube. Ethyl alcohol, 95 per cent, is added up to 8 c.c. (1 c.c. of fluid and up to 4 c.c. with alcohol may be used and gives a better reading where the protein content is greatly increased). The contents are then acidulated with a drop of 10 per cent acetic acid or just a trace of glacial acetic acid. The contents are then heated carefully to boiling over a Bunsen burner. The protein immediately flocculates. The contents of the centrifuge tube are transferred to a vaccine tube with the capillary tip graduated in 0.01, 0.02, 0.03, 0.04 and 0.05 c.c. and centrifuged until the precipitate is all thrown down into the capillary tip. Some of the precipitate may collect on the sides, then the supernatant solution is stirred with a glass rod and the contents centrifuged again.

The amount of total protein normally present in 2 c.c. of cerebrospinal fluid as determined by this method is from 0.005 to 0.015 c.c. or from 25 to 75 mg. per 100 c.c. We have been using 2 c.c. of cerebrospinal fluid because this amount gives a large quantity of precipitate and a better volumetric reading unless the protein content is greatly increased; then 1 c.c. is used. The method is simple, practical and clinically accurate. The determination can usually be completed with three minutes when centrifuged at the rate of 3,000 revolutions per minute. The reagents are simple and no elaborate apparatus or preparation of standards are required. The vaccine tubes can be procured from any supply house making laboratory glassware. The precipitate is readily removed from the capillary tip by using a capillary tip pipette with a rubber bulb from a medicine dropper fastened to the large end.

0.01	c.c. or	50 mg. per 100 c.c.	Normal
0.015	c.c. or	75 mg. per 100 c.c.	High Normal
0.02	c.c. or	100 mg. per 100 c.c.	
0.03	c.c. or	150 mg. per 100 c.c.	
0.04	c.c. or	200 mg. per 100 c.c.	
0.05	c.c. or	250 mg. per 100 c.c.	

The authors draw the following conclusions from their observations:

Normal fluids contain 25 to 75 mg. per 100 c.c. or 0.005 to 0.015 c.c. in 2 c.c.

BLOOD STAINING: The Influence of the Hydrogen-Ion Concentration on Blood Staining, Mommsen, H. Klin. Wchnschr., May 7, 1926, xix, 844.

The author concludes that the zone which gives good results in an appropriate staining time is between P_H 6.0 and 7.0.

For daily use the author recommends the following mixture of a nearly neutral buffering mixture of even parts of primary and secondary phosphate.

m/1 phosphoric acid	6.7
m/1 sodium hydroxide	10.0
Distilled water	ad. 1000.0

The electrometric examination of a Giemsa solution prepared with this mixture gave P_H 6.93. The author did not choose the medial of the favorable zone he found, but the alkaline pole, because in higher P_H the staining time is shorter. By preparatory staining in Jenner's or May-Grunwald's method, the purplish tint of the eosinophiles is avoided.

The staining technic is the following:

The fixation lasts three minutes and the preparatory staining after May-Grunwald in the usual way four minutes. For the Giemsa staining one drop of Gruebler solution is taken for 1 c.c. of phosphate buffer. It lasts five to ten minutes. Distilled water is used for rinsing. In the preparatory staining the author omits the buffering, because he had experienced that it is disadvantageous with P_H 6.93. Furthermore he rinses with distilled water, because the theoretically correct rinsing with the phosphate buffer is practically negligible at P_H 6.93.

RABIES: Eliminating a Source of Error in the Laboratory Diagnosis of Rabies, Borman, E. K. Am. Jour. Pub. Health, May, 1926, xvi, 476.

The following method was devised to eliminate red blood cells as sources of possible error.

Dissect out the brain. Make impression and smears from Ammon's horn, cerebellum, and cerebral cortex in the usual manner. The layer of tissue upon each slide should be made as thin as possible, for a thick layer is more easily washed or rubbed away with subsequent treatment.

Place the slides in the following solution:

Methyl alcohol (C. P.)	98 c.c.
Glacial acetic acid	2 c.c.

Allow the slides to stand in this solution for three minutes. Dry quickly over a flame, taking care to avoid intense heating. Transfer the slides to a 10 per cent aqueous solution of potassium carbonate and allow them to stand thus for five minutes. Wash in a very gentle stream of tap water. Dry by gently blotting them with smooth absorbent paper. Care must be taken not to wash or rub away any of the tissue adhering to the slides.

Flood the slides with the following dry mixture:

Methylene blue (saturated aqueous sol.)	3 drops
Basic fuchsin (saturated alcoholic sol.)	2 drops
Tap water	20 c.c.

Warm the slides by passing them through a flame once. Allow them to stain for not more than one minute. The staining time will depend upon the purity and solubility of the dyes used.

Wash the slides in a stream of tap water, dry, and examine for Negri bodies.

Any stain which will demonstrate the presence of Negri bodies may be used. The foregoing stain, which is used in many laboratories, has, however, given the most consistent results with this method. This stain should be reddish-blue in color; the red dye should not dominate the blue. It must be kept at ice box temperature when not in use. It should never be used after standing for more than twenty-four hours, as it deteriorates rapidly.

The attempt was made to remove the red cells by the use of acetic acid after the slides had been fixed in C. P. methyl alcohol. The results so obtained were variable.

By employing a mixture of the two substances the complete destruction of the red cells is effected simultaneously with the fixation of the essential parts of the brain tissue.

The use of the acetic acid produces a change in the staining properties of the tissue, chiefly characterized by a diminished affinity for the dyes used. Other acids have this same general effect upon the tissue, so that it may be ascribed to a change in hydrogen-ion concentration of the tissue proteins. The carbonate solution is employed to offset this factor by a process of neutralization.

If the large nerve cells are a clear blue and the matrix a dull red, it is an indication of excessive treatment with the acid-fixative or of insufficient treatment with the carbonate solution. Negri bodies are difficult to perceive under such conditions as they are stained but faintly.

A slide properly prepared by this method should show the large nerve cells with reddish-blue cytoplasm and deep blue nuclei in a matrix of brilliant red. Negri bodies if present will then show a characteristic red with typical granular structure. Unstained, hole-like areas will be perceived where red blood cells were located.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Newer Knowledge of Nutrition**

THE most comprehensive exposition of our actual scientific knowledge of nutrition and deficiency diseases which the reviewer has as yet read. Food deficiencies and deficiency diseases necessarily assume a prominent part in the discussion for it is from a study of these diseases especially that we have gained greatest knowledge of nutritional needs.

Throughout the work the "practical application aspect" has been kept to the fore.

Clinical Laboratory Procedure†

THIS volume is written primarily for the practicing physician in an effort to facilitate the performance of routine laboratory analyses. To this end the author has described and illustrated many ingenious homemade articles of apparatus which reduce the cost of laboratory work considerably without sacrificing accuracy.

Only the usually accepted routine studies are described in detail. Prominence is given to the interpretation of findings. We note with pleasure that the minutiae of the more highly technical procedures such as the Wassermann reaction, the colloidal gold reaction, procedures which the physician himself will not do, are omitted and that this space is applied to much better advantage in a discussion of the clinical interpretation of these reactions.

The final chapter consists of a list of the most common diseases with the laboratory studies which are indicated in each. This is very brief but should be of distinct service to the busy practitioner.

Blood Chemistry—Colorimetric Methods‡

THE second edition of Dr. Stone's volume follows quite closely the lines of the first. The author limits himself strictly to his text. He does not incorporate alternative methods but uses only those which have been more universally accepted throughout the country. Furthermore he only describes the tests for those substances which are of interest to the practical clinician. We note with pleasure that he has elaborated somewhat on the sections devoted to clinical interpretation of results. The volume covers only a small restricted field but this it does in a very acceptable manner.

*The Newer Knowledge of Nutrition. The Use of Foods For the Preservation of Vitality and Health. By E. V. McCollum, Ph.D., Sc.D., and Nina Simmonds, Sc.D. Cloth. Illustrated. Pp. 675. The Macmillan Company, New York.

†A Manual of Clinical Laboratory Procedure—For the Use of the General Practitioner. By Robert A. Kilduffe, A.B., A.M., M.D. Cloth. Illustrated. Pp. 287. The C. V. Mosby Company, St. Louis, Mo., 1926.

‡Blood Chemistry—Colorimetric Methods—for the General Practitioner with Clinical Comments and Dietary Suggestions. By Willard J. Stone, B.Sc., M.D. Cloth. Illustrated. Pp. 129. Price $3.25. Paul B. Hoeber, Inc., N. Y., 1926.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

He has included a few dietaries in an appendix. We hope that in his next edition he will extend this so that appropriate sample dietaries will be found corresponding to each of the series of laboratory investigations.

*A Manual of Normal Physical Signs**

THIS is a tabloid manual for the use of the tyro in physical diagnosis. Students should find it of considerable assistance as a corollary to didactic instruction.

It should be of even greater use to instructors in elementary physical diagnosis as a framework or guide on which to develop a scheme of instruction. The book cannot serve as a substitute either for textbooks or for collateral reading.

Potter's Therapeutics, Materia Medica and Pharmacy†

THE fourteenth edition of a volume as well known as this scarcely requires an introduction. The make-up follows that of past editions but has been brought strictly up to date. Facility in use as a reference volume is favored by the use of thumb indexes.

The work covers the classification and the administration of medicines, a lengthy materia medica with remarks on the physiologic action and therapeusis of the various drugs, discussion on pharmacy and prescription writing, a long chapter on special therapeutics, alphabetically arranged by disease, which should be of value as a reference manual to the physician, and, in the appendices, Latin terminologies, various tables, and summaries of the existing narcotic and prohibition regulations.

*A Manual of Normal Physical Signs. By Wyndham B. Blanton, B.A., M.A., M.D. Cloth. Pp. 215. The C. V. Mosby Co., St. Louis, Mo., 1926.

†Therapeutics, Materia Medica and Pharmacy. By Sam'l O. L. Potter, A.M., M.D., M.R.C.P., Lon. Revised by R. J. E. Scott, M.A., B.C.L., M.D. Cloth. Pp. 972. P. Blakiston's Sons & Co., Philadelphia, Pa., 1926.

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EDITORIALS

Laboratory Examinations—Not Laboratory Tests

IT IS greatly to be regretted, for many reasons, that the crowded condition of the present-day medical curriculum prevents more than a general outline of many of the things which it attempts to cover, and forces into the consideration of the mechanism, pathology, and diagnosis of disease, a composite picture of the typical case.

It is, perhaps, for this reason and because of the impression thus gained, that there is a perceptible degree of confusion as concerns the relation of laboratory examinations to diagnosis.

Too many men, for example, are accustomed to regard the Widal agglutination test as a test for the presence or absence of typhoid fever.

It is, of course, nothing of the sort but merely a method devised for the demonstration of the presence or absence of agglutinins for the typhoid bacillus and this information has nothing to do with the presence of typhoid fever, and is of no diagnostic significance whatsoever until it has been correlated with all the other findings in the case.

It is obvious that the reaction of an individual to disease is a manifestation dependent upon and influenced by two interacting factors: (a) the character and degree of the stimulation exerted, and (b) the ability of the particular individual to react.

The methods of the laboratory are devised to detect or measure the character and the degree of reaction, and *the results always require interpretation!*

When a leucocyte and differential count are made in the presence of a suspected inflammatory process, one is not making a *test* for the presence of appendicitis, for example, but an examination to detect evidences of reaction to an inflammatory process, just as the temperature and pulse are taken as a part of a study for evidences of reaction to pathologic processes. And, just as there are no pathognomonic temperatures, so there are, with only occasional exceptions (as in the leucemias), no pathognomonic leucocyte counts.

The sole aim and object of laboratory studies in general is to conduct such examinations as shall serve as a source of information to be acquired in no other way. The meaning or interpretation of the findings is elicited only when they are compared, correlated, and evaluated in conjunction with all the other data, historic, clinical, roentgenologic, etc., obtained by all the available means at hand.

If laboratory procedures are consistently thought of as methods of examination rather than as tests for various diseases there will be less clinical confusion in their application and interpretation.

—*R. A. K.*